American Academy of Allergy, Tenth Annual Meeting, Shamrock Hotel, Houston, Texas, February 1, 2, 3, 1954.

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ALLERGY ABSTRACTS

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Vol. 24, No. 5, September, 1953. The Journal of Allergy is published Bi-Monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription Price: United States, its Possessions, Pan-American Countries, $8.50; in Canada and other foreign countries, $9.00. Entered as Second-Class Matter, November 15, 1929, at the Post Office at St. Louis, Missouri, under the Act of March 3, 1879. Printed in the U. S. A.


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# The Journal of Allergy

VOL. 24 SEPTEMBER, 1953 No. 5


## Original Articles

### ALLERGIC REACTIONS TO PENICILLIN

A PANEL DISCUSSION


WILLIAM P. BOGER, M.D., WILLIAM B. SHERMAN, M.D.,
IRVING W. SCHILLER, M.D., SHEPPARD SIEGAL, M.D., AND BRAM ROSE, M.D.

EDITED BY IRVING W. SCHILLER, M.D., BOSTON, MASS.


*Dr. Rose.*—The panel of experts who will discuss the subject and answer any questions are Dr. Sheppard Siegal and Dr. William Sherman, of New York, Dr. Irving Schiller, of Boston, and Dr. William Boger, of Philadelphia.

Dr. Sherman, will you open the discussion by describing the common manifestations?

*Dr. Sherman.*—I am going to start off by reviewing very briefly some of the common reactions, most of which are already very familiar to you. Probably the commonest type of reaction to penicillin, which occurs certainly in 1 or 2 per cent of all patients receiving penicillin, is the delayed urticarial type or what is often spoken of as the "serum-sickness" type of reaction. I think the term "serum-sickness" type is very applicable because it certainly can imitate practically all of the usual features of a reaction to heterologous serum. Like ordinary serum-sickness, it varies in severity from a relatively mild and brief urticaria to an extremely severe illness which may last for several weeks. The predominant feature is urticaria and angioneurotic edema. It is rather characteristic that the palms and the soles of the feet are likely to be involved by the urticaria. Associated with the urticaria, in the majority of cases, there is arthralgia and swelling of the joints, particularly the small joints of the hands and feet, often a really typical arthritis of these joints. Many of these patients have fever. The fever may be negligible in a mild case or it may be as high as 105° in a severe case. As you can see, all of this description exactly fits ordinary serum-sickness and the two conditions are so similar that if a patient with some injury has received tetanus antitoxin and penicillin simultaneously, the reaction may


Presented at the Annual Meeting of the American Academy of Allergy, Feb. 26, 1953, Boston, Mass.

Received for publication April 15, 1953.

be such that one is hard put to say which of the two agents has been the cause. Like serum-sickness, this type of reaction has a characteristic incubation period of five to perhaps fourteen days. I would not attempt to say what is the longest possible incubation period, but certainly anything up to two weeks is not unusual. As in serum-sickness, we assume that this delayed reaction and incubation period indicate that the patient is developing sensitivity as a result of the administration of the drug. Fig. 1 illustrates a few

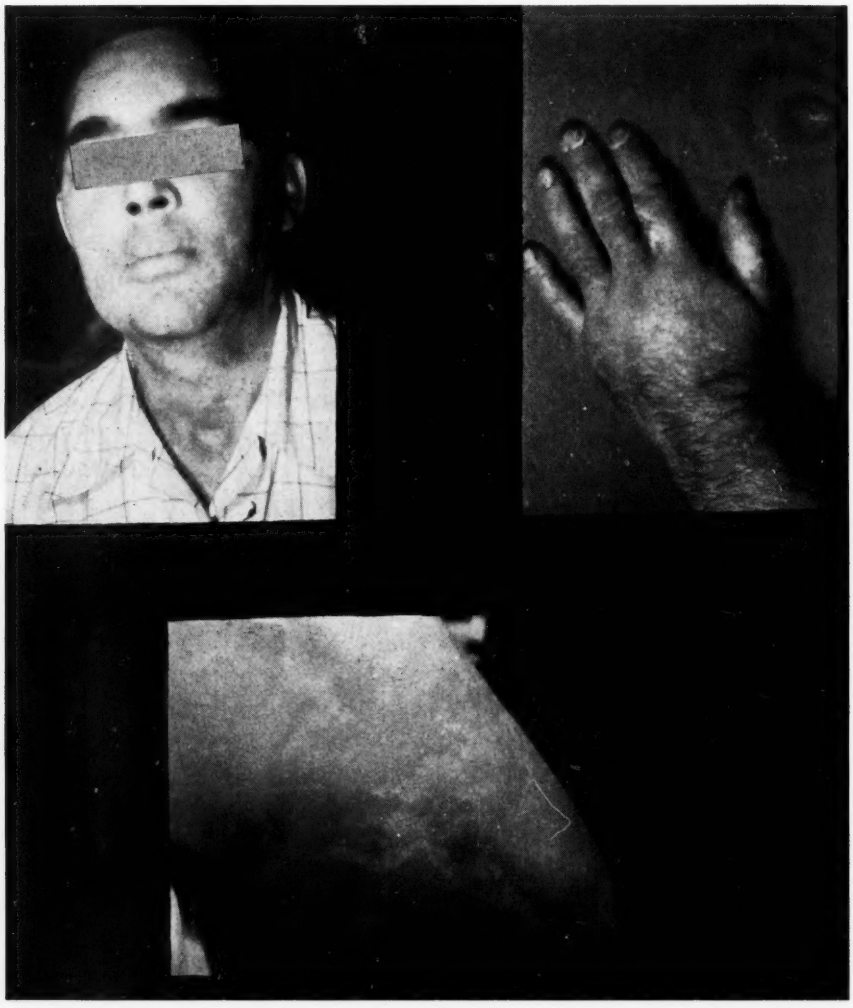

Fig. 1.—Serum-sickness type of reaction to penicillin manifested by edema of the face, swelling and stiffness of fingers, and confluent urticaria of trunk and limbs.

of the characteristic features of this type of reaction—massive and often confluent urticaria involving often a large part of the body surface, edema of the face, particularly about the eyes, and swelling of the hand, including particularly the small joints of the fingers. This picture could equally well be one of serum-sickness due to heterologous serum.

Of the various types of penicillin which have been used, crystalline penicillin and procaine penicillin seem to produce essentially the same form and incidence of urticarial reactions. However, one type of penicillin, penicillin in beeswax and oil, produced an unusual incidence of this type of reaction, often associated with severe reactions at the sites of injections. Fig. 2 shows the arm of a patient who had received one injection of penicillin in oil and wax in the left deltoid region about nine days before the photograph was made. The main feature shown in this illustration is a large local reaction at the site of the injection. With penicillin in oil and wax, there are two differences from the procaine penicillin and the crystalline penicillin: first, the incidence of sensitization is much greater, which we may assume is due to the fact that the oil and wax act as adjuvants increasing the tendency to sensitization. Second, the absorption of antigens from the site of injection is much delayed so that when sensitization occurs, the local reaction is very great.

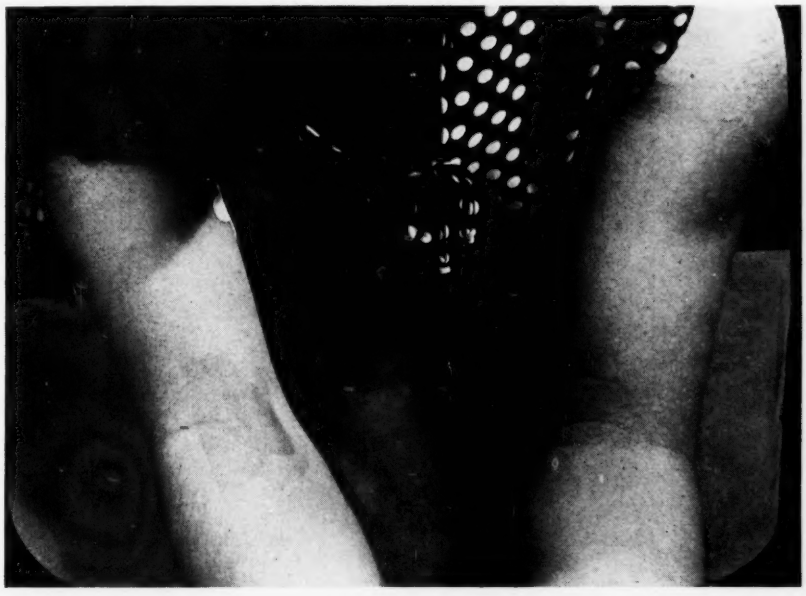

Fig. 2.—Local reaction occurring eight days after injection of penicillin in oil and wax into left deltoid region.

Fig. 3 shows a side view of the same patient and one of the interesting features is that over the site of injection is not only a large edematous swelling but a localized area of vesicular dermatitis. In other words, this patient is showing two distinct types of reactions at the same time, the dermatitic type and the urticarial type. Such local reactions are very much less common with procaine penicillin than with penicillin in oil and beeswax.

Another relatively common type of reaction, which is less often seen nowadays with procaine penicillin than formerly, is what has been known as the "id" type of reaction which, at least superficially, resembles an attack of epidermophytosis involving particularly the interdigital spaces, the groin

and flexion creases. This type of reaction characteristically occurs early in the course of treatment with an incubation period of a few hours to not more than one day. The reaction is characterized by redness, oozing, and itching. It has been suggested repeatedly that this type of reaction represents a cross reaction in a patient who, became of a previous fungus infection of the skin, has a local sensitization to fungus products, which gives a cross reaction with penicillin. It has been pointed out that patients showing this type of reaction are very likely to show positive reactions to skin tests with trichophyton or oïdiomycin. However, so many patients show reactions to these fungus antigens that we are unable to use these tests as a criterion for treatment; otherwise, we would needlessly omit using penicillin in many cases.

Fig. 3.—Local reaction to injection of penicillin in oil and wax showing vesicular lesion superimposed upon subcutaneous edema.

A third common type of reaction is dermatitis medicamentosa, a relatively nondescript generalized rash, which may be macular or papular, quite similar to what may be seen after the sulfonamide drugs, phenobarbital, or numerous other agents. This type of reaction varies tremendously in severity. It may be very mild and not infrequently treatment has been continued, in spite of the occurrence of the reaction, with relatively little difficulty. Certain of these nondescript rashes, which we lump together as dermatitis medicamentosa, may become increasingly severe and progress to a serious and disabling exfoliative dermatitis which may persist for a long time after the penicillin is discontinued.

The fourth common type of penicillin reaction is contact dermatitis due to topical application of penicillin, usually in the form of penicillin ointment. This is a typical vesicular contact dermatitis with the usual features. The senstization is usually developed in the course of one to two weeks after the first contact with the ointment, so that it is practically never an immediate reaction in the patient who has not previously been exposed. This type of reaction is illustrated by Fig. 4, showing a patient who had a small area of follicular infection in the bearded part of his face and was given penicillin ointment to apply. He applied it regularly and, after a week or so, the area of irritation spread. He kept applying more and more as the reaction spread so that what started as a small area about the size of a dime came to involve the entire neck and lower face. The rash was now of the vesicular type quite similar to poison ivy, a typical contact dermatitis.

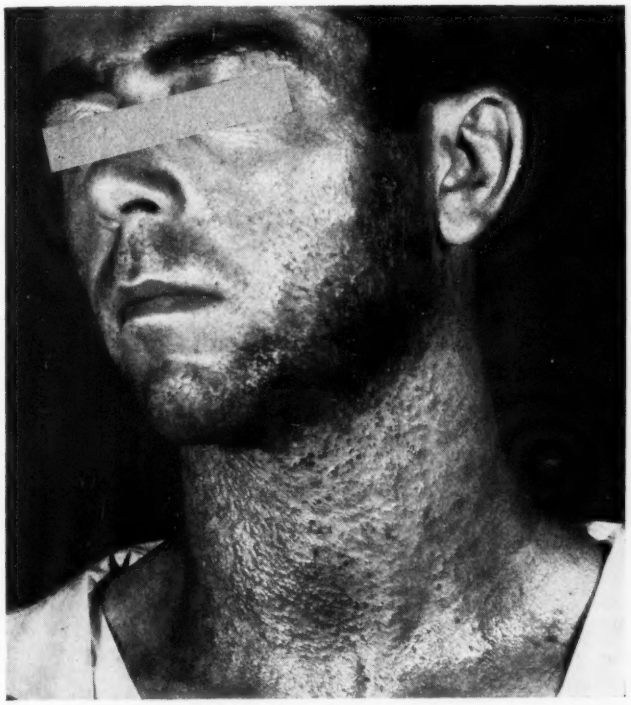

Fig. 4.—Contact dermatitis due to penicillin ointment.

*Dr. Rose.*—Dr. Sherman has just outlined the common types to you. I am now going to ask Dr. Boger, unless there are any further comments from the panel, to talk about the more infrequent manifestations of penicillin reactions.

*Dr. Boger.*—A good beginning point for the discussion of the rare and rather serious reactions to penicillin is a slide that Dr. Schiller was kind enough to bring along. This female patient (Fig. 5) shows an extensive

purpura, and Dr. Schiller advises that the patient's purpura was nonthrombocytopenic in nature. Others have observed purpura due to sensitivity to penicillin that has been accompanied by thrombocytopenia. The manifestation of purpura serves to emphasize the role of vascular permeability in the development of various of the rare sensitivity phenomena. Some of the commoner manifestations, itching, urticaria, and rashes, that have just been described can be explained, at least in part, by vascular permeability. It is an easy step in our thinking to go from the purpura just shown to an occasional microscopic and even gross hematuria if one conceives a purpuric type of lesion in the kidney due to penicillin sensitivity. Vascular permeability and damage to small blood vessels can be involved as an explanation of the arthritis and hydrarthrosis that are seen in cases of penicillin hypersensitivity classified as serum-sickness. In rare cases of serum-sickness the

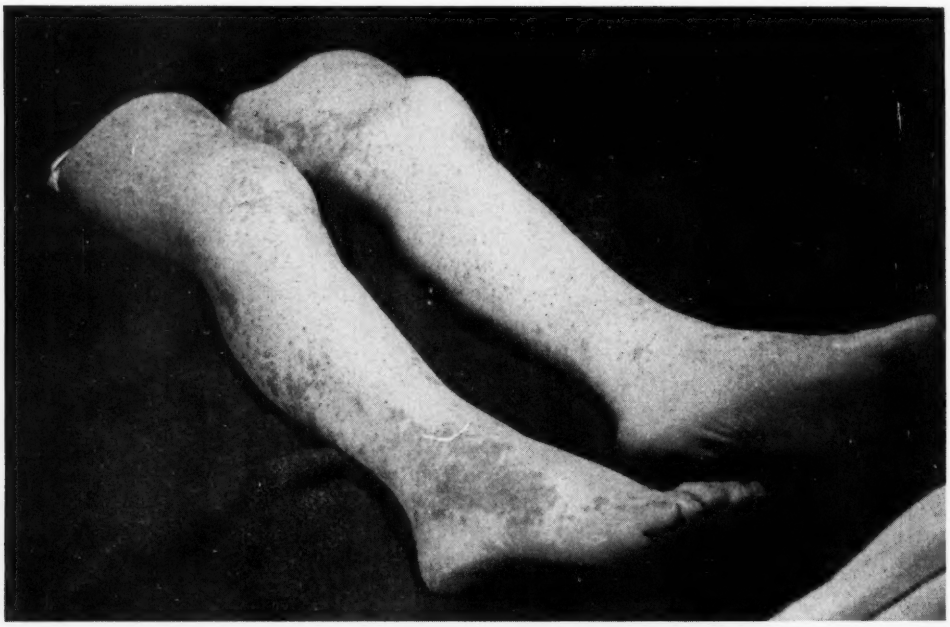

Fig. 5.—Purpuric eruption following the administration of penicillin.

joint symptoms persist for three and four months and we have followed several such patients. Indeed, the syndromes that have been described under the designation of "collagen diseases" are being reported as due to penicillin sensitivity. It has taken a number of years to develop the full picture of penicillin reactions, but it also took a number of years, during which the sulfonamide drugs were employed, to show that erythema nodosum was a mild manifestation and periarteritis nodosa the ultimate. That fatal arteritis can result from the administration of sulfonamide drugs has been amply confirmed and we are beginning to hear of obliterative arteritis and generalized periarteritis nodosa due to penicillin. There are those investigators who

claim to be seeing photosensitivity and even lupus erythematosus due to penicillin. Personally, I have not seen such a case, but perhaps one of the other panel members can speak more familiarly on this point. Angioneurotic edema with obstruction to the airway is uncommon but distressing; exfoliative dermatitis, syncope, collapse, and death are not common but they have all been reported. It seems reasonable to suggest that all the phenomena of drug sensitivity, including the gamut of symptoms assigned to the various collagen diseases, will be shown, sooner or later, to be due to hypersensitivity to penicillin.

The practice of introducing penicillin intrathecally should be mentioned. When the nervous system is exposed directly to penicillin in a quantity as little as 50,000 units, severe convulsions can result.[12] This is due to direct chemical irritation and has nothing to do with allergy. Death has been caused by the intrathecal administration of larger doses of water-soluble penicillin and, consequently, there is an increasing trend away from this mode of administration of the drug.

All of the rare reactions to penicillin have not been reviewed for I think any phenomenon that has been observed in connection with hypersensitivity can be observed in connection with penicillin administration. We have tried to emphasize some of the more serious and rare complications in the hope of avoiding the use of penicillin, or any other drug, if it is not indicated.

*Dr. Rose.*—You will see that as we progress the reactions become more severe. We will now ask Dr. Siegal, therefore, to say something of the anaphylactic and fatal cases which appear to be on the increase.

*Dr. Siegal.*—Penicillin anaphylaxis, it seems to me, is becoming a more frequent problem to which we will have to give very serious attention. As an indication of this may I point out that in the past fifteen months 15 cases of penicillin anaphylaxis were published, and, of these, 6 were fatal. By contrast from 1946 to 1951, there were only six cases reported with but one fatality.* Moreover, in the past year or so I know of nine unreported cases occurring in the area of New York and vicinity alone, and, of these, four were fatal. Several of these recent cases, reported or unreported, have been reactions to crystalline penicillin G and in others where aqueous suspension of procaine penicillin was used all the evidence incriminates penicillin rather than procaine. On January 31 of this year there were published in the *Journal of the American Medical Association*[1] six cases of anaphylactic collapse from penicillin, one of which was fatal. Most recently, my attention was called to a report of anaphylactic death from penicillin taking place on board a ship of the United States Navy.[2]

At the May meeting of the New York Allergy Society in 1952, I first reported three cases of penicillin anaphylaxis, later published in THE JOURNAL

*In view of the more frequent use of penicillin it is difficult to say whether or not this greater number of reported anaphylactic reactions actually represents an increased incidence. All cases reported as having this type of reaction have been patients who have received previous courses of penicillin. As the use of penicillin increases, obviously more patients will receive second and third courses of the drug.

OF ALLERGY.[3] Two of these were hospital residents who reacted with severe shock, to crystalline penicillin G in one case, to procaine penicillin G in the other. There was reason to believe that both were atopic individuals, one being a hay fever sufferer. Whereas fortunately they recovered, the third patient, a 67-year-old asthmatic woman, had a fatal reaction to an intramuscular injection of 300,000 units of aqueous procaine penicillin. She almost immediately felt a strange taste in her mouth, complained of inability

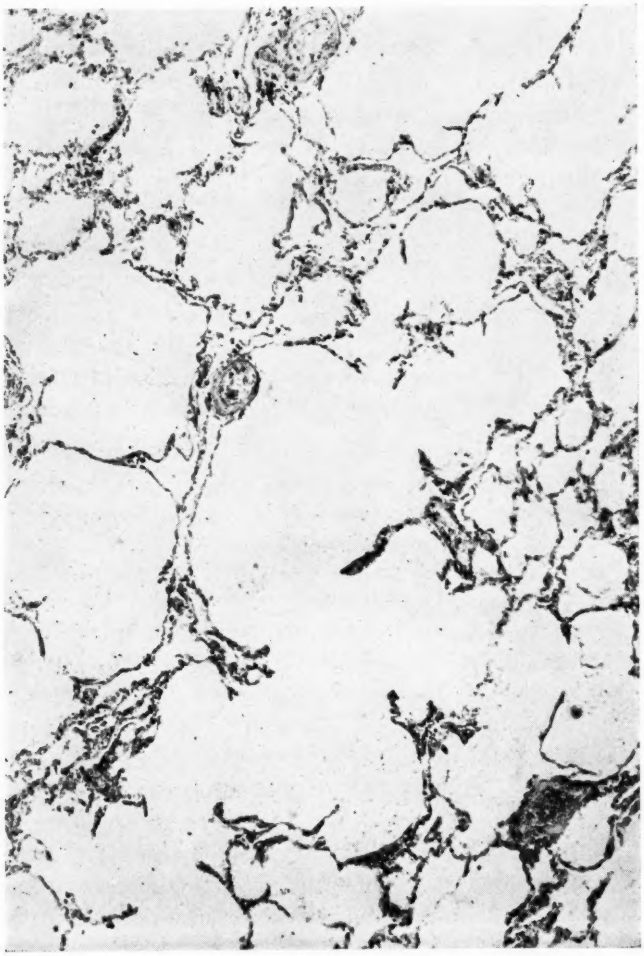

Fig. 6.—Penicillin anaphylaxis in an asthmatic patient. Acute pulmonary emphysema showing distended alveoli.

to breathe, promptly became unconscious, and was in collapse. She was dead in the doctor's office before anything could be done. Autopsy was not performed. It is important to note that two of the three patients gave no history of a previous reaction to penicillin, while the third had previous reactions which were ignored.

Of great interest are two autopsied cases of penicillin anaphylaxis reported by Curphey, Medical Examiner of Nassau County. Both were asthmatics who died in a few minutes following procaine penicillin in one instance and after combined penicillin-streptomycin in the other. At post-

Fig. 7.—Penicillin anaphylaxis in an asthmatic patient. Extensive bronchial and bronchiolar mucoid exudate filling many alveoli.

mortem the striking findings in the first case were the voluminously distended lungs of acute emphysema, the so-called "guinea pig" lungs (Fig. 6). In the second case the chief finding was of marked mucoid secretion in the bronchi, shown on microscopic section to extend into the alveoli (Fig. 7).*

I have the distinct impression that with repeated exposure to penicillin through the years, largely by injection, increasing numbers of persons are becoming sensitized to the anaphylactic level. Penicillin can no longer be

*I am grateful to Dr. Theodore J. Curphey for permission to use these photomicrographs.

regarded as largely an innocuous drug. How are we going to prevent these extremely dangerous, even fatal reactions? It seems to me that the following points should be noted:

1. Anaphylaxis occurs only in those who have had penicillin previously, usually on several occasions.

2. Anaphylaxis can occur even though penicillin was well tolerated previously.

3. It occurs more frequently in allergic individuals and is more likely to be fatal in asthmatics.

4. The dangerous and potentially shocking dose is usually the first injection following a prolonged interval since the last exposure to penicillin. This interval has often been one of several months. This is comparable to what is observed in animal anaphylaxis. Therefore, all precautions must be taken for this initial injection. Subsequent successive doses are probably not dangerous.

I would like to suggest certain precautions for the prevention of anaphylaxis:

1. Avoid needless sensitization through the use of penicillin for trivial or doubtful indications. Other forms of chemotherapy may be preferable.

2. For mild infections employ oral penicillin which is certainly less likely to cause fatal reactions.

3. A careful history as to previous penicillin treatment and reactions is important. Previous accelerated and above all immediate reactions are especially significant.

4. Modification of the technique of penicillin injections is an additional precaution. The initial or potentially shocking dose should be given into the arm rather than into the buttock so that a tourniquet may be applied. Avoid scrupulously the possibility of accidental intravenous injections. It may be wise, following an earlier suggestion of Waldbott, to inject the first drop or two, then pause for forty-five seconds before completing the injection, as the really violent reactions may become evident within seconds. Keep epinephrine directly at hand when giving this injection of penicillin.

5. Preliminary skin tests may be helpful in screening out some individuals who may react with anaphylaxis. A simple scratch test has been helpful and should be done routinely. Earlier experience with penicillin skin testing has been with aqueous solution of crystalline G penicillin. More recently, however, aqueous procaine penicillin has been used effectively. In view of the much greater convenience to the general physician, it is suggested that the scratch test may be carried out with the treatment material ready at hand, aqueous procaine penicillin, 300,000 units per cubic centimeter, adding a drop of water at the skin site to prevent too rapid drying. It is best to wait at least fifteen minutes before making a final reading. Intracutaneous tests may be dangerous and are best carried out only after a negative scratch test. A strongly positive skin test reaction should rule out penicillin administration. Since allergy and even anaphylaxis to penicillin may occur in the absence

of a positive skin test, an additional helpful precaution might well be the oral administration of a 50,000 unit penicillin tablet ten minutes before the intramuscular injection.

*Dr. Boger.*—May I make a comment? The severe and even fatal reactions that are being cited should be viewed in their proper perspective. No one will disagree that penicillin is an allergen with sensitizing potential, but we should not lose sight of the rarity of serious reactions if viewed in terms of the enormous quantity of penicillin that has safely been administered and of the countless number of lives that have been saved. Penicillin is the foremost of the antibiotic agents. Its indiscriminate use is unfortunate and is to be deplored.

*Dr. Rose.*—Dr. Schiller is going to discuss the use of skin tests because we do want to know what can be done first of all to tell whether a patient is sensitive to penicillin and what we can do about it. I would like to ask Dr. Schiller also to tell something about the interpretation of the skin tests.

*Dr. Schiller.*—You are all familiar, I am sure, with the scratch, intracutaneous, and patch tests used to study penicillin sensitivity. Fresh aqueous solutions of penicillin G, or penicillin O if you wish, in a strength of 10,000 units per milliliter are prepared. All tests should be properly controlled using the diluent as the blank solution. We test patients initially by the *scratch method,* for a few serious reactions have been reported following intracutaneous tests. A small drop of the test solution, as well as a drop of the control solution, is applied to the skin and then the skin is scratched through the drop. A positive reaction is characterized by a wheal and erythema which appears within fifteen or twenty minutes, or sooner. The wheal may be pseudopodic, may vary from 10 to even 30 mm. or more in diameter, and itching may be present. This, of course, is compared with the control site, which should appear normal except for perhaps the slight trauma of the scratch. The scratch test, if negative, is followed, in our clinic, by the *intracutaneous test* which is carried out by injecting 0.05 ml. of the 10,000 unit strength intracutaneously. The site is observed for fifteen or twenty minutes and when positive is also a typical wheal and flare reaction. In any case, whether the reaction is positive or negative, the site of the injection is observed for the next forty-eight hours for the appearance of the *delayed positive reaction.* When this occurs, its appearance is similar to a positive tuberculin reaction. Here one sees an erythematous area which may measure from 10 to 20 mm. or more in diameter, frequently raised and indurated and oftentimes studded with papules. The reaction may continue to grow in intensity up to seventy-two hours and then may persist, frequently, for a week or longer, leaving a fading brownish site in its wake. The *patch test* is done by making a solution of 50,000 units per milliliter or 100,000 units per milliliter of penicillin, applied to the skin soaked in a piece of cotton, and read in forty-eight hours. When the reaction is positive, one sees an erythematous area in which vesicles of varying size are present.

Now, what is the clinical significance of these various tests? In spite of the well-known limitations of skin tests and the fact that their reliability is still open to question, we feel that they serve to call attention to possible disastrous effects that may follow the administration of penicillin. A negative reaction certainly does not rule out the possibility of a serious reaction, nor does a positive one indicate that an immediate systemic reaction, or even a reaction of the delayed type, will ensue with penicillin therapy. Nevertheless, we can speculate that had some individuals been skin tested with penicillin and the results given proper consideration, collapse or anaphylactic reactions might have been avoided. A positive scratch test in an individual with a history of having had one or more courses of penicillin or one suggesting an allergic reaction from penicillin certainly is indicative of clinical sensitivity and the likelihood of a severe reaction should penicillin be given. Likewise, the immediate positive intracutaneous test should suggest the existence of a high degree of penicillin allergy. The delayed positive reaction or "spontaneous" penicillin sensitivity, first described by Welch and Rostenberg,[4, 5] is said to be present in about 4 or 5 per cent of individuals who have never received penicillin. It has been suggested that such sensitivity possibly has developed from subtle exposure to related fungus products or previous fungus infections of the skin. This needs further clarification. The incidence of the delayed reaction is increased in patients who have had penicillin in the past and is much higher in patients who have had penicillin reactions. I am sure that both Dr. Siegal and Dr. Boger will have something to say about this. Patients with the delayed positive type of reaction are not perhaps as likely to have manifestations of the immediate type. If clinical sensitivity develops at all, it would take the form of the more common urticarial or "serum-sickness" type of reaction. Recently we saw a patient who is an exception to this and we will have more to say about this later. So far as the patch test goes, we have had but little experience with it, and I hope that the other members of the panel will have more to say about it. It seems that the patch test is of only questionable value as an index of general penicillin sensitivity.[6]

*Dr. Rose.*—There is the question, of course, that is always raised with reference to the intradermal test with penicillin. I know of one case where death occurred very quickly following the use of this test in the hands of a capable physician who was trained in allergy. Scratch testing, I believe, is to be preferred; however, I would like to hear further opinions about it.

*Dr. Boger.*—Pertinent to Dr. Schiller's remarks, we have observed generalized rash and fever from intracutaneous skin tests performed during our comparisons of one penicillin with another. Each test constituted 2,000 units of the penicillin in question and two tests on 4,000 units (approximately 2.5 mg.) produced generalized reactions on an allergic basis.

*Dr. Rose.*—The technique of skin testing has been described, and a general interpretation given. Skin testing has its limitations, however, and I would like to ask Dr. Sherman to discuss this point.

*Dr. Sherman.*—I do not want to take a great deal of time because I think that most of this subject has been covered. I would like to emphasize the fact that a negative reaction to scratch test or intracutaneous test is never a reliable index that the patient is not sensitive, that he or she will not have an immediate reaction, or that a delayed reaction will not occur. As far as the delayed urticarial type of reaction goes, I do not believe that any skin test is of any value. Oftentimes during and immediately after the delayed urticarial reaction, intracutaneous tests, even going up to considerably higher levels than have been mentioned previously, are completely negative. When positive reactions are obtained, they are almost always so small that they are not significantly different from what may be obtained in normal individuals. However, it is obvious, I am sure, to all allergists that in those rare cases where you do get a strong, immediate urticarial reaction to either a scratch or intracutaneous test, you have a very unusual degree of sensitivity and the greatest possible danger if ordinary doses of penicillin are given in spite of them. I do not have anything worth while to say either in regard to delayed reactions to intracutaneous tests or to patch tests.

*Dr. Rose.*—I know that Dr. Siegal has something further to say about delayed skin reactions and we are going to ask him to tell us about it.

*Dr. Siegal.*—Quite a few years ago, when we studied the delayed intracutaneous test, we found that among 25 cases of the "serum-sickness" type of reaction, 10 had a positive test of the twenty-four to forty-eight hour type. Subsequently, Risman and Boger[7] published a series of observations on the several penicillins about which I know Dr. Boger will speak, and in which, incidentally, there is statistically demonstrated the fact that the delayed test does actually indicate the existence of penicillin allergy. Fifty-five per cent of the patients who had a previous reaction to penicillin gave a positive delayed test as compared with only a 10 per cent incidence among those who had penicillin previously but without a reaction.

*Dr. Schiller.*—Before the subject of skin tests is left, I might mention that for the past three months we have been interested in the incidence of penicillin sensitivity in the allergic population passing through our clinic. Intracutaneous tests were carried out comparing penicillin G and O. Of some 50-odd patients studied, the majority of whom had previously received penicillin, no positive immediate reactions nor delayed reactions were seen. However, when subjects were selected (hospital personnel) for study who had had previous penicillin reactions, the story was quite different. These frequently had immediate or delayed positive cutaneous tests. It seems to us that the incidence of positive penicillin skin tests correlates best with past systemic reactions to the antibiotic.

*Dr. Rose.*—Since we should try to know something about the basic immunology of the reactions, I would like to ask Dr. Sherman what he would say about that.

*Dr. Sherman.*—The first thing I would say is that *nothing* is really known about the immunology and that all of the ordinary rules that you might expect to apply seem to be remarkably unreliable in relation to penicillin reactions. Now to go back to what I have spoken of as the "serum-sickness" type of reaction, this so closely resembles serum-sickness due to heterologous serum that anyone might reasonably expect that all the same immunologic mechanisms would be apparent. However, there is, as I have stated, no reliable immediate urticarial reaction to skin tests during or after the reaction. The delayed type of reaction or the immediate intracutaneous test is occasionally positive, but the percentage is not high enough to make it of any great significance as to interpretation. The attempts to demonstrate antibodies of any type in the serum of patients having this type of reaction have been pretty uniformly negative. Whether we attempt to do this by passive sensitization of human skin, by Dale reaction in guinea pigs, or any other method including precipitin, there is no reliable immunologic test for this "serum-sickness" type of reaction, whereas, of course, when the same clinical type of reaction is due to heterologous serum, all of these tests are fairly consistently positive. Now another feature that is very strikingly different in serum-sickness and in the penicillin reactions is that it is only occasionally that the occurrence of penicillin urticaria leads to a persistent anaphylactic type of sensitization in which a subsequent dose will produce an immediate reaction. It happens often enough so that there is certain danger involved, but it is nowhere near as consistently positive as in the case of heterologous serum. It is very common, indeed, to find that a patient who has had one delayed urticarial reaction to penicillin, on the next course of penicillin several months later will have another delayed urticarial reaction with the same incubation period, which would lead you to believe that the patient was again starting to become sensitized with the first dose. However, if such patients are treated the second time the incidence of the delayed reaction is much greater in those who have previously had a delayed reaction. I do not think that there is any doubt at all that the anaphylactic type of reaction Dr. Siegal has described is often associated with positive skin tests and is often associated with a demonstrable Prausnitz-Kustner type of antibody. Without doubt it occurs more often in patients who show a family or past history of allergy so that it is the one exceptional type which appears to fit in with our ordinary concepts of sensitization.

*Dr. Rose.*—Dr. Schiller has a slide which he would like to show.

*Dr. Schiller.*—First, I would like to state a few facts about the patient before showing the slide. He is S. F., a 58-year-old man who has had bronchial asthma for about seventeen years. In the past eighteen months his asthma had increased in severity and, because we were unable to control it adequately, he was given several short courses of oral cortisone with beneficial effects. Because we will have something to say about this later, we would have you remember that when he was tested intracutaneously

and by inhalation to house dust extract he showed significant reactions, and, incidentally, he has been receiving weekly injections of house dust extract.

During December, 1951, at which time he had not been on cortisone for about three months, he developed a respiratory infection and a purulent sinusitis and because of this was given 400,000 units of procaine penicillin intramuscularly. He had had penicillin in the past on a number of occasions with no untoward reactions. Within a matter of a minute or less, he appeared flushed, was dyspneic, and had marked asthma. Urticarial wheals were seen over the chest and abdomen. He was the picture of a typical constitutional reaction. Within ten minutes following the administration of several small doses of Adrenalin, the reaction subsided.

TABLE I. TESTS WITH PENICILLIN

| (S. F., 57, W., M.) (BRONCHIAL ASTHMA) | | | OCTOBER 1952 | NOVEMBER | JANUARY | FEBRUARY 1953 |
|---|---|---|---|---|---|---|
| Skin test | "G" | Immediate | 0 | 0 | +++ | +++ |
| | | Delayed | +++ | +++ | + | 0 |
| | "O" | Immediate | 0 | 0 | +++ | ++ |
| | | Delayed | +++ | ++ | ± | 0 |
| Passive transfer | "G" | | | 0 | | 0 |
| | "O" | | | 0 | | 0 |

In October, 1952, about eleven months later, S. F. was skin-tested with several penicillins and these results are shown in Table I. These tests were done intracutaneously using 0.05 ml. of the 10,000 unit per cubic centimeter strength. You can see that the immediate intracutaneous tests were negative to both penicillins G and O but the delayed reactions, observed forty-eight hours later, were markedly positive. These reactions were raised, indurated, quite red and itchy. The reactions to both penicillin G and O were equal in size and measured about 25 mm. in diameter. S. F. was retested in November and showed the same picture—negative immediate reactions and positive delayed reactions. At this time blood was drawn for passive transfer studies but passive transfer antibody was not detected. Results of retests in January were somewhat surprising in that the patient had immediate reactions to both penicillin G and O and the delayed reactions were markedly decreased. When last tested in February, 1953, the immediate reaction was again found to both G and O, somewhat less to O, but at this time he showed no delayed reactions. Passive transfer studies at this time were again negative. One other point should be made. In the interval that we were studying S. F.'s skin response to the various penicillins, we had another patient who had had a "serum-sickness" like reaction about nine days following the administration of intramuscular penicillin. This was a patient of Dr. F. C. Lowell's and we will refer to him as W. M. About two weeks after W. M. had received his injection of penicillin, when his urticarial reaction was subsiding, he had, when tested intracutaneously with penicillin, an immediate reaction with a 20 mm. wheal and erythema. A delayed reaction was not observed. At that time blood was drawn from W. M. for passive transfer

studies and S. F., the patient who has just been described to you, acted as a recipient. This was November, 1952, when you will recall S. F. had no passive transfer antibody in his serum and showed only delayed positive reactions. When the site in S. F. (which held W. M.'s serum) was challenged with penicillin, there developed an immediate wheal and erythema which was pseudopodic and about 20 or 25 mm. in diameter, a definite passive transfer reaction. This indicated that S. F.'s skin was capable of reacting in the same manner as W. M.'s if he had only developed the appropriate type of antibody. We know that S. F. was able to react this way because he did so to house dust. We were interested in following this passive transfer reaction for the next forty-eight hours to see whether or not he would have a delayed reaction. At the site where the passive transfer reaction occurred, a delayed reaction developed which was far less intense than a control site which was injected with only penicillin. In discussing this response at a recent meeting,[8] Dr. Lowell brought up several interesting possibilities. First, one might say that the antibody in W. M.'s serum might have neutralized the penicillin, thereby bringing about a less intense reaction. Second, since the reaction involves the production of a wheal and the accumulation of a lot of fluid in the area, penicillin may have simply been diluted. Finally, the skin having reacted quite intensely with a wheal and erythema may become nonspecifically desensitized and was therefore unable to react with the violence that it did previously. This recalls the observation that in patients who exhibit the immediate reaction to tuberculin or purified protein derivative, the reaction at forty-eight to seventy-two hours is usually absent or, if present, is of small degree. I think this is a very interesting sequence of events and there may be other possibilities to explain it.

Perhaps Dr. Sherman would like to comment on the immunologic setup present in the case we have just mentioned.

*Dr. Sherman.*—I think this is an exceedingly interesting case. I do not know how often this type of thing occurs, but this is one thing that does have some analogy in relation to sensitization to heterologous serum because at least occasionally the earliest stage of sensitization to heterologous serum is associated with a delayed reaction and the immediate reaction occurs subsequently. However, I think that we have no way of knowing that this situation will be observed often and I do not believe you want to go away with the impression that a delayed reaction to penicillin is the forerunner of the immediate reaction because I am sure that a great many patients have been tested repeatedly over a period of months or years and have always shown delayed reactions and never shown immediate reactions. However, I think we can at least speculate that this is a patient who is in danger of having the anaphylactic type of reaction and we can only say that we do not know how often this may occur.

*Dr. Boger.*—I want to agree wholeheartedly with Dr. Sherman's observations. There are no infallible rules covering the phenomena of hypersensitivity to penicillin. Our inability to predict what will happen is

attested to by the following experience. A patient was given 1.2 million units of procaine penicillin suspended in aluminum monostearate and oil gel and two weeks later he had as near to an exfoliative dermatitis as I care to see. We despaired of the man's life but he recovered and was discharged from the hospital. Unbeknown to those of us who went through this harrowing experience, the man came back to the antisyphilitic clinic and he was given another 1.2 million units of procaine penicillin suspended in oil and aluminum monostearate gel. I was all ready to take up residence in the nearest penal institution offering luxury accomodations, but to our amazement and my relief the man did not even turn a hair. This experience convinced me that you cannot predict anything with regard to penicillin reactions.

TABLE II. SKIN SENSITIVITY TO PENICILLIN

| | GROUP A NO KNOWN PENICILLIN THERAPY | | GROUP B PREVIOUS TREATMENT WITH PENICILLIN | | GROUP C KNOWN REACTION TO PENICILLIN | | TOTALS | |
|---|---|---|---|---|---|---|---|---|
| RESPONSE TO SKIN TEST | NO. OF PATIENTS | % | NO. OF PATIENTS | % | NO. OF PATIENTS | % | NO. OF PATIENTS | % |
| +G, +BT | 6 of 137 | 4.5 | 8 of 113 | 7.1 | 14 of 26 | 53.5 | 28 of 276 | 10.0 |
| +G, +O | 2 of 93 | 2.2 | 11 of 110 | 10.0 | 12 of 21 | 55.0 | 25 of 224 | 11.2 |

*Dr. Rose.*—That gives you some idea of the complexity of this enormous subject. In order that we may complicate it a little more, before we actually come to the period of treatment, I am going to ask Dr. Boger to speak again and this time discuss the reactions to the various types of penicillin, such as "G," "O," and "BT."

*Dr. Boger.*—It has been stated earlier that an individual's past penicillin experience is significant. You will observe (Table II) that in Group A, with no known penicillin experience, there was 2.2 per cent reactivity; in Group B, with known penicillin experience—oral, intramuscular, repository or otherwise—a 10 per cent reactivity. In Group C, those patients who had shown allergic responses, 55 per cent reacted to intracutaneous skin test. With regard to the supposedly hypoallergenic penicillin O, these observations should not be interpreted to mean that all patients sensitive to G also react to O, for some patients sensitive to G have been reported to tolerate O and in such cases penicillin O has extreme value. The skin tests were read at a half-hour and again at twenty-four and at forty-eight hours. No test was regarded as positive unless erythema and induration measured at least 4 mm. in two diameters.

BT penicillin is an unavailable biosynthetic penicillin similar to O, and the experience with it parallels that with O (Table II). With no previous penicillin therapy, there was a 4.5 per cent reactivity. This is an original response for which we have no explanation other than the existence of a common antigen shared by penicillin and a variety of naturally occurring fungi. The exposure of the patient to this common antigen may be in the

form of unrecognized fungus infection or ingestion of fungi and their products. In Group B, out of 113 with known penicillin experience, there was 7.1 per cent reactivity; in the small group with previous known reactivity, the incidence was 53.5 per cent. Those observations are submitted only to show that one's past penicillin does have some bearing on the skin sensitivity.[7] A patient with subacute bacterial endocarditis during the second week of therapy with water-soluble penicillin G developed high fever and reactivation of the bacteremia. We were desirous of substituting penicillin O in order that penicillin therapy could be continued, but by skin test the patient reacted just as violently to O as to G.

In a discussion of hypoallergenic penicillins, it is appropriate to mention l-ephenamine penicillin G ("Compenamine"). The same claim for lesser potential of sensitization has been made for this preparation as for penicillin O, but regrettably our own experience is not extensive enough to comment on this point. In connection with a discussion of hypoallergenic penicillin it seems proper to mention that although we may feed chemical precursors to microorganisms producing penicillin in an attempt to produce a penicillin that is not normally produced and so overcome the sensitization problem, Nature cannot be made to forget completely her natural manufacturing methods. Hence, according to all the information that has come to my attention, penicillins BT and O contain a small amount of penicillin G as a contaminant.

While efforts have gone forward to produce new penicillins with less sensitizing potential, other efforts have been made to alter the penicillin molecule with the idea of accentuating certain valuable properties. Procaine penicillin by combining a local anesthetic and penicillin G has given us a repository or long-acting preparation. To date there is no convincing evidence that the procaine portion of the molecule has occasioned any additional sensitization, but we should be alert to this possibility. Penethamate ("Neo-Penil") has been shown to diffuse more readily into lymph glands, the respiratory tract, and the central nervous system, than penicillin G itself. These established advantages of this new penicillin are probably negated by the serious side-effects which have been recently reported. Benzethacil ("Bicillin" or "Permapen") represents a chemical modification of penicillin which results in an insoluble penicillin that gives prolonged plasma concentrations after intramuscular injection and this type of penicillin will probably find a considerable use in the treatment of syphilis.

All of the new penicillins represent a chemical union of two drugs and as such the potentials of sensitization are increased. In this connection attention is called to the current practice of administering combinations of penicillin and sulfonamides, penicillin and salicylates, penicillin and probenecid, and penicillin and streptomycin. It should be borne in mind that all of such combinations of penicillin with other drugs increase the likelihood of the patient being sensitized to one or all of the components of these combinations. In view of the grave potentials of sensitization in terms of serious side-effects, our use of penicillin should be as discriminating as possible.

*Dr. Rose.*—Now, apparently, there may be another way of testing for penicillin sensitivity and I am going to let Dr. Schiller tell you something about that.

*Dr. Schiller.*—Inhalation tests using penicillin as an aerosol were carried out comparing the reactivity of the bronchial tree or lung to the inhalation of penicillin G and O. The patient studied is the one described earlier as S. F., whose skin tests with penicillin G and O, you will recall, were of the delayed type which, subsequently, became immediate. I would call your attention

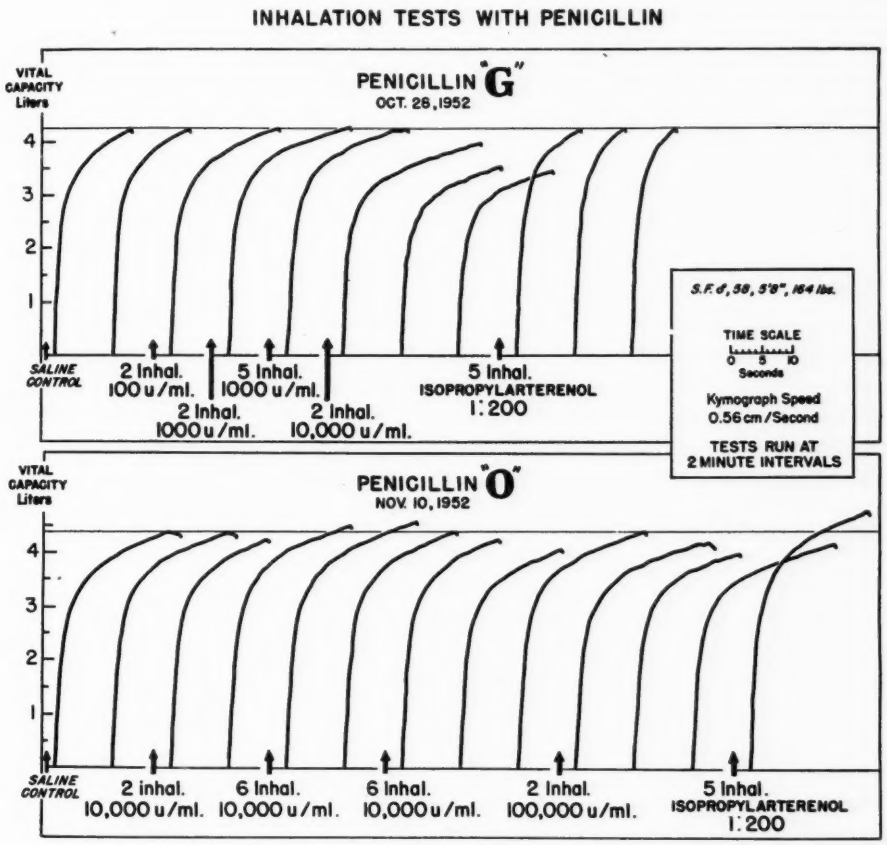


Fig. 8.

now to the upper portion of Fig. 8 which shows the expiratory tracings of S. F.'s vital capacities recorded on a moving drum. This experiment on October 28 utilized penicillin G as the test aerosol. The patient's control vital capacities were in the vicinity of 4 liters and showed no change after the inhalation of a saline control. Vital capacities were performed at about one and one-half to two-minute intervals. Following two inhalations of the 100-unit strength aerosol and again following a total of seven inhalations of the 1,000 unit solution, the vital capacities remained essentially at the

normal control value, though examination of the tracings indicates that the patient was having some difficulty in getting air out of his lungs. The careful measurement of the volume expired in the first second indicates this. He was then given two inhalations of the 10,000 unit strength of penicillin aerosol and, as you can see, within a minute or two thereafter there was a sharp fall in vital capacity as well as a marked decrease in the rate of expiration. It is interesting that during this period he experienced little or no asthmatic symptoms. Since we felt that we had a very significant response, we did not wait any longer and gave him five inhalations of isopropylarterenol 1:200 and, as you can see, his vital capacity returned to normal. The patient was again tested on November 11 in the same way but was exposed to an aerosol of penicillin O. This is seen in the lower portion of Fig. 8. After inhalation of the saline control he had a total of fourteen inhalations of the 10,000 unit strength with only a very equivocal response, which very shortly returned to normal control value. After two inhalations of the 100,000 unit strength there again was a suggestion of a pulmonary response but, in contrast to penicillin G, this was not significant. Note the tenfold increase in concentration of the penicillin O aerosol. With five inhalations of isopropylarterenol his vital capacity exceeded the control value. We think, then, that this man might have tolerated penicillin O, but that is purely speculative. There is one other interesting point that I would like to make: at the time these inhalation tests were done, the patient exhibited only the delayed positive type of skin reaction. This is the first patient in our experience, and we have been testing patients by the inhalation method since 1946, who has had an immediate pulmonary response in the absence of the immediate type skin test.

*Dr. Boger.*—This is a very definitive technique for showing sensitivity, namely, inhalation of the allergen, but for my part I choose to have nothing to do with such a means of determining reactivity. In this connection I have two most disturbing recollections. One person who loves the experimental method and will not listen or learn from the experience of others used an inhaler that was sent to him during that short period of time when penicillin dust in the nostrils was enjoying some degree of acceptance. This "investigator" narrowly escaped a tracheotomy, for he developed severe angioneurotic edema with involvement of the larynx. A child who received penicillin nose drops was not so fortunate; he had to have a tracheotomy.

Laryngeal edema is something I have tremendous respect for, and I want no penicillin in my nasopharynx.

*Dr. Schiller.*—In answering Dr. Boger, this is not the time or place to go into the discussion of the merits of the inhalation test. I would only refer him to the articles on the subject that have come out of our laboratory in the past five or six years.[9, 10] The method is simple and innocuous. If I were allowed to do but one test, and one test only, on a patient with bronchial asthma for specific diagnostic purposes, the choice would be the inhalation test. What we did with this patient just described bears no resemblance to the inhalation

of penicillin powder (quantitatively, the difference is vast), and we would agree with Dr. Boger that the inhalation of such powdered penicillin is potentially dangerous in anyone who is sensitive to the drug.

*Dr. Rose.*—I think we ought to have several very short words about the treatment of these penicillin reactions; I am going to ask each member of the panel to brief you on that subject.

*Dr. Siegal.*—I do not think I have anything very new or important to say on the subject. Some of the patients, of course, who have severe reactions simply will not respond to ordinary antihistamines. I have found that phenergan is probably the single most effective antihistamine for the urticarial type of reaction. When this fails I think all of us, sooner or later, rely upon ACTH or cortisone. I would like to mention one thing. Occasionally we see patients who have very prolonged reactions of urticaria following penicillin. I personally have seen three lasting from five to six months, and Epstein in *California Medicine*[11] has recorded one of fifteen months' duration following penicillin. In such cases, if these measures fail, desensitization with penicillin is well worth trying and has been successful in my hands.

*Dr. Sherman.*—I do not think there is anything to add to what Dr. Siegal has said. In the immediate anaphylactic type of reaction, of course, the treatment is that of any anaphylactic reaction with Adrenalin the main reliance. In the severe urticarial type of reaction, in my experience, the antihistaminic drugs have not been very satisfactory. When cortisone or ACTH became available we very quickly came to use these. I think that essentially all the other cutaneous reactions that we have mentioned, including exfoliative dermatitis, respond better to ACTH and cortisone than to any other drugs available.

*Dr. Schiller.*—The antihistaminic drugs may be helpful in relieving both the pruritus and severity of the urticaria seen in the course of the serum-sickness-like syndrome in penicillin allergy. However, these agents have little or no effect on the joint pains, lymphadenopathy, or renal disturbances. In severe cases, we have found cortisone to be very effective in controlling both the skin and joint manifestations. I think the treatment of the immediate reaction has been well covered.

*Dr. Boger.*—Epinephrine is of real value in the ordinary case, but ACTH or cortisone may be mandatory for severe cases. A 5 per cent solution of glucose containing 10 mg. of ACTH is dripped in intravenously over an eight-hour period. This dose may be repeated twice or three times during the twenty-four hour period. In some instances it may be necessary to duplicate this treatment on the second day. ACTH used intravenously has five to seven times the potency of intramuscularly administrated drug and we feel that ACTH acts faster and is more physiologic than cortisone. ACTH and cortisone are the best answer for the problem of severe reactions that have followed penicillin administration.

*Dr. Rose.*—I will not attempt to summarize this excellent exposition on penicillin sensitivity in any detail. Perhaps it is sufficient to emphasize the importance of these reactions primarily from the incidence of reactions of a fatal nature. There are undoubtedly many more reactions than have been reported in the literature, and I personally am aware of three, fortunately not my own cases. The variety of nonfatal responses is of extreme interest to anyone with a training in allergy, and it may be that further careful study of the skin reactions may elucidate basic allergic mechanisms.

I would add a word regarding treatment. We have used the intravenous administration of hydrocortone with success in one case of near-fatal shock. This compound, 100 mg. in 500 ml. of 5 per cent dextrose, acts quickly and is probably the most rapid acting of all hormone preparations. Since penicillin is still one of the most useful antibiotics at our disposal, let us continue to use it carefully and wisely.

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# UNUSUAL PENICILLIN HYPERSENSITIVENESS

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THE widespread employment of penicillin in medicine has disclosed many side reactions of a disturbing, often serious nature. About 5 per cent of normal people react unfavorably to this comparatively new drug. At the meeting of the American Academy of Allergy in Boston, Feb. 26, 1953, a panel discussion was devoted to the hazards of penicillin therapy, and although cognizance was taken of ten fatal cases resulting from penicillin therapy reported in the literature and in the experience of the members, the exquisite sensitiveness that sometimes occurs and that was exhibited in the case here reported was not greatly emphasized.

## CASE REPORT

A 35-year-old white drug salesman was referred to us because of known penicillin hypersensitiveness. While in the Army in 1945 he had been wounded and treated with large doses of penicillin over a period of ten days for a bone infection. Subsequent to this he had received, almost annually, injections of penicillin for nasal stuffiness associated with infection of the nose and sinuses, and on several occasions penicillin aerosol. Two years ago (1951) following a penicillin injection he vomited immediately, but exhibited no other untoward effects. He thinks he had penicillin the year after that without any reaction. On Jan. 28, 1952, he took by mouth four Biosulfa tablets.* Within a period of minutes he was in violent anaphylactic shock from which he was rescued by heroic measures including hospitalization. One month later while in the office of a doctor who was treating another patient with penicillin aerosol in a room about thirty feet away, he suddenly developed the initial symptoms of shock, and saved himself from more serious manifestation by leaving the premises immediately. After this, on two or three occasions, in a drugstore, he developed rhinorrhea and distressing tightness of the chest when the druggist was changing penicillin tablets from one bottle to another. On the last occasion he did not get relief until Adrenalin was administered. During and prior to the development of these reactions he had suffered chronically from a stuffy nose, rhinorrhea, and headaches which his physician had treated successfully on an allergic basis, regarding him after skin testing as sensitive to certain foods and inhalants.

When seen by us for the first time, Oct. 28, 1952, this patient appeared as a healthy young adult, six feet in height, and weighing 190 pounds. Temperature was 98° F.; pulse was 80; respiration 20; blood pressure 140/88. Eyes, ears, nose, and throat were normal. Skin was free from eruptions; the neck, the thorax, the heart, and the lungs were negative; the abdomen, back, and extremities were all normal. Laboratory studies revealed a red blood count of 5,620,000 and a hemoglobin level of 15 Gm.; the white blood count was 7,650 with 53 per cent neutrophils, 17 per cent eosinophils, and 30 per cent lymphocytes. The sedimentation rate was 3 mm. in one hour; nasal blowings showed no eosinophils; the serologic test for syphilis was negative and skin tests to a number of ingestants and inhalants, except for several mild reactions, were negative.

The patient was asked to wait in an examining room. The door was closed. In another room some distance away penicillin G was given by aerosol to another patient. In a few minutes, without knowing what was going on in the other part of the building, the patient reported in this article developed severe paroxysmal dyspnea with coughing, wheezing, and rhinorrhea which did not respond to adrenalin until intravenous aminophylline was given.

Received for publication, June 1, 1953.

*A proprietary combination of crystallin penicillin G, sulfadiazine, sulfamerazine, and sulfamethazine manufactured by The Upjohn Company.

The next day in order to determine whether this patient was sensitive also to penicillin O, serial dilutions were made of penicillin O for patch testing. The patches consisted of white blotting paper 1 cm. square prepared in the usual way. Three were employed saturated with 10 units, 100 units, and 1000 units of penicillin O, respectively. All three reacted promptly producing itching and wheals. The largest wheal with pseudopods measured 4 by 5 cm. The development of sneezing, cough, and a sense of constriction in the chest caused the removal of the patches and the washing of the areas with soap and water within twenty minutes of the time of the application of the patches.

Hyposensitization was regarded as inadvisable, and the patient was dismissed with the advice that he discontinue the detailing of all preparations containing penicillin, that he carry with him always an ampule of adrenalin and a sterile syringe, and that he have prepared a metal tag bearing the inscription that the wearer is hypersensitive to penicillin and that it should be given him under no circumstances. This tag he was advised to wear on all occasions.

## SUMMARY AND CONCLUSIONS

Several features of this case invite comment:

1. There is great value in a history carefully taken concerning the previous use of penicillin and the patient's experience after its employment.

2. Danger lurks not only in the administration of penicillin therapeutically but in the actual testing for the hypersensitiveness that is suspected.

3. Extreme sensitiveness may at times exist in patients allergic to penicillin producing a situation in which skin testing by the scratch or intracutaneous method might well prove fatal.

4. Patch testing with graduated dilutions of penicillin may serve as an immediate warning of danger in the oral or parenteral use of the drug. It is safer than either the scratch or intracutaneous method, and in suspected cases should be substituted for, or precede, the other conventional tests.

5. This individual was sensitive both to penicillin G and to penicillin O so that one could not be substituted for the other.

6. An individual exhibiting this degree of sensitiveness should wear continuously an indestructible tag about the neck or on a wrist chain as a warning in case of accident attended by unconsciousness against the risk of having administered to him this otherwise useful antibiotic.

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# ANAPHYLACTIC REACTION FROM TOPICAL PENICILLIN

## Report of a Case

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THE primary purpose of this paper is to report a case of immediate reaction following the antral instillation of penicillin. The literature contains only one other report describing two similar cases of penicillin reactions.[1] Doubtless otolaryngologists have encountered others which remain hitherto unreported. Any inferences arising from the discussion of this case constitute nothing new, but simply a review of some of the more clinically important and related aspects of penicillin sensitivity.

### CASE REPORT

I. K., a 30-year-old white female hospital attendant, was treated at the Cushing Veterans Administration Hospital, Framingham, Mass., in December of 1946 for a right antral sinusitis. About a year later an antrostomy was performed because of a flare-up. On both occasions she was given a series of intramuscular injections of procaine penicillin. On July 30, 1951, an antrostomy was performed on the left side for a draining sinus which followed the extraction of an abscessed tooth. On this occasion she was placed on oral penicillin. On the fifth day of oral penicillin she developed mild tingling of the palms of the hands, soles of the feet, and vulvar region. At this point the patient discontinued the penicillin of her own accord.

On Aug. 8, 1951, she reported to the E.N.T. Section of the Boston Veterans Administration Regional Office for further treatment. On this date the left antrum was irrigated with saline solution with no untoward reaction and the patient was placed on Aureomycin therapy. Several days later, and because of essentially no improvement, 100,000 units of crystalline penicillin G in 2 ml. of distilled water was instilled into the left antrum through the antrostomy opening. Within a matter of seconds she complained of nausea, weakness and faintness, burning and itching of the hands and feet and generalized tingling. She sneezed several times. Wheezing of a mild degree was present. Beginning with the hands, her entire body soon became erythematous and edematous. The conjunctivae were injected and chemotic. The pulse was somewhat weak and rapid at a rate of 160; the blood pressure, 80/60. At no time was she pale and sweaty nor did she lose consciousness. Within several minutes after the onset of the reaction 0.5 ml. of epinephrine hydrochloride 1:1,000 was administered hypodermically and this was followed by 100 mg. of tripelennamine hydrochloride administered orally. Within an hour all symptoms gradually disappeared and the patient felt quite normal.

The patient's family and past history were devoid of any atopic background. There was no history or evidence of any mycotic disease of the skin. Routine intradermal tests for inhalants, pollens, and molds disclosed a borderline reaction to house dust and Alternaria. On Sept. 13, 1951, skin tests were performed with crystalline penicillin G taken from the same batch of penicillin that produced the reaction. The scratch test was quite

From the Allergy Section of the Regional Office, Veterans Administration.

Presented at the Ninth Annual Meeting of the American Academy of Allergy, Boston, Mass., Feb. 28, 1953.

Received for publication Feb. 12, 1953.

positive in a dilution of 10,000 units per ml. A wheal with pseudopods and measuring about 3 cm. in diameter and with a marked flare was obtained by intracutaneous testing with 0.02 ml. of solution containing 1,000 units per ml. A drop of solution, containing 50,000 units per ml., and applied directly to the skin, had to be wiped away because of immediate itching and erythema. The delayed reaction, read in forty-eight hours, was also positive, the papule measuring 1.4 cm. in diameter. The trichophyton test was not done. An attempt to demonstrate circulating antibodies on two normal recipients proved unsuccessful. Fourteen months later this patient was retested in connection with comparative studies on penicillin G and O and found to be negative to the immediate and delayed reactions of both penicillins. Inhalation aerosol with both penicillins failed to produce bronchospasm or any diminution in vital capacity.

## DISCUSSION

The pattern of this case closely parallels the two cases reported by Everett.[1] In both his cases the reaction following antral instillation of penicillin was virtually immediate. The reaction in the first case, a nonatopic, was apparently the first indication of penicillin sensitivity. The reaction in the second case, an atopic asthmatic, had been preceded by mild itching associated with both oral and aerosol penicillin. Again, in both cases, there had been previous antral instillations with penicillin. Unfortunately, it was not possible definitely to ascertain from either the patient or the hospital records whether or not penicillin had been instilled into the sinus at any time prior to the reaction. Such information might well be of primary interest to the otolaryngologist. In all three cases the reactions were preceded by recent administration of penicillin in one form or another, thus bearing out the observation of Keefer[2] that the closer the intervals between administration the greater the likelihood of reaction. All three cases had repeated courses of penicillin. Keefer[2] and Long[3] have pointed out that such a situation serves to increase the potentiality of sensitization.

Everett's[1] first case was associated with marked bronchospasm, severe circulatory depression, and no dermal reaction (urticaria). Higgins and Rothchild,[4] in reporting a case of fatal anaphylactic shock from procaine penicillin which more closely resembles animal anaphylaxis, also reviewed previously reported anaphylactic reactions to penicillin. They point out that Everett's[1] second case, associated with bronchospasm and dermal reaction but no shock-like symptoms, did not constitute a true anaphylactic reaction. Depending on one's definition of anaphylactic reaction in the human being, one might seriously question as to whether or not the reaction reported could correctly be termed as anaphylactic. Burleson,[5] in describing his case of wheezing, dermal reaction, and shock following intramuscular procaine penicillin, used the term "anaphylactoid." This case was also preceded by several courses of penicillin in the past followed subsequently with oral penicillin, the latter producing the first indication of sensitivity, namely, slight itching. Irwin, Graham, Jacobson, and Burrage[6] referred to their case as one "of a shock-like type of reaction" associated with an "atopic type of penicillin sensitivity." For purposes of more accurate description it would appear more suitable to label this reaction as a severe immediate allergic reaction associated with some de-

gree of shocklike symptoms. It is conceivable, however, that had not epinephrine been administered so promptly, more imposing shocklike symptoms might have supervened.

It is not uncommon, as seen in this case, for both the immediate and delayed skin tests to become negative at some future time after the reaction. Penicillin, with its amino acid-like structure, is regarded as a poor antigen. McClosky[7] suggests that its low molecular weight may account for its irregular ability to act antigenically. It is commonly believed that haptene formation plays the larger role in penicillin reactions particularly in the nonatopic individual. It is noteworthy that, in the case of Irwin and associates,[6] atopic in character, with marked skin reactions and the presence of circulating antibodies, both the immediate and delayed tests also turned negative some months later.[8] Peck and his co-workers[9] have demonstrated the unreliability of the skin test and that not infrequently reactions develop in individuals negative to both the immediate and delayed skin test. Everett[1] made no reference to skin tests but, again, it is noteworthy that his first patient experienced a profound degree of shock from intramuscular penicillin administered inadvertently by another physician several months after the first reaction.

Considering the widespread use of penicillin these past ten years, the literature contains very few reports of the so-called "anaphylactic" and more immediate types of reactions. A recent editorial[10] in THE JOURNAL OF ALLERGY points out that there have been many more fatal cases and alarming reactions which have not been reported. Keefer[2] states that the greatest incidence of sensitization arises from topical administration and the least from the oral route. As more people receive an increasing number of penicillin courses and subsequently take oral penicillin, oral sensitization may assume greater importance. The symptoms are usually mild in character such as slight tingling or itching of the hands and feet and are frequently overlooked by the patient or disregarded by the physician. Doubtless many alarming reactions and even some deaths have ensued during the course of treatment with agents capable of producing anaphylactic reactions at a time when the physician failed to pay attention to that slight itching of the palms or other premonitory symptoms. The history of mild sensitivity associated with oral ingestion of penicillin was not obtained in this patient chiefly because of the patient's lack of understanding of the term "sensitivity." One author of a recent paper on penicillin reactions expostulated in this regard that the patient should be questioned in a language that could be clearly understood.

## SUMMARY

1. A case of severe immediate allergic reaction following the antral instillation of penicillin has been presented.

2. Two other similar cases noted in the literature have been reviewed.

3. The use of the term "anaphylactic" and some of the related aspects of penicillin sensitivity have been discussed.

4. More careful attention should be paid to the patient's pre-existing sensitivities in the use of penicillin.

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# THE ETIOLOGICAL ROLE OF STREPTOCOCCUS TOXIN-ANTITOXIN SYSTEMS IN THE EXPERIMENTAL PRODUCTION OF ARTERIAL SENSITIZATION

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## INTRODUCTION

THE association of streptococcal infections with lesions of the cardiovascular system has long been recognized. On the basis of clinical, laboratory, and pathologic findings, evidence has been accumulating to implicate a possible etiological role for the streptococcus in certain cardiovascular lesions of man suspected of arising as a result of hypersensitivity or immune mechanisms. There is cause for more than just suspicion in the relationship of Group A $\beta$ *Streptococcus hemolyticus* to rheumatic fever according to data that may be found summarized elsewhere in several excellent reviews.[1-4] Periarteritis nodosa, often considered to be a lesion of vascular hypersensitivity, has been reported in association with rheumatic heart disease[5] and also following scarlet fever,[6-8] a disease caused by streptococcus erythrogenic toxin. The association of streptococcus has also been suspected in some cases of disseminated lupus erythematosis.[9] Consequently the problem has been further approached from the experimental standpoint by attempting to utilize the streptococcus in inducing pathologic cardiovascular lesions in laboratory animals. This has been successfully accomplished in mice, guinea pigs, or rabbits by employing injections of living or killed streptococcal broth cultures,[10] whole streptococci or their component fractions,[11] streptococcus toxin,[12-20] streptococcus plus foreign proteins[21-23]; by employing streptococci in Arthus[24] and Shwartzman reaction techniques[25]; and by inducing cutaneous[26, 27] and pulmonary[28] streptococcus infections.

An analysis of the results of these experimental studies is not altogether conclusive. Some of these investigators felt that the lesions resulted from toxic manifestations,[11, 15] nonspecific reactions,[10, 29] or "streptococcal injury."[17-18] Though it has been shown that streptococci,[30] their somatic component fractions,[31, 32] and streptococcus toxin[20, 33] are capable of giving rise to in vivo reactions of hypersensitivity in laboratory animals, and although a study of the experimental conditions, techniques employed, and results obtained in other studies concerned with the experimental production of cardiovascular lesions would suggest that the mechanisms of sensitization to the streptococcus or its products played a major role, there is no definite or unequivocal proof of this.

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This investigation was supported (in part) by a Research Grant from the National Heart Institute, of the National Institutes of Health, Public Health Service.

Received for publication March 4, 1953.

Criep and Mayer[19] were unable to demonstrate serum precipitins in animals showing cardiovascular lesions as a result of injections with streptococcus toxin, and other investigators make no mention of studies to detect circulating antibodies against the streptococcus, its somatic fractions, or extracellular components in their series of experimental animals. There has been no correlation offered relating the appearance, type, organ or tissue specificity, and histologic details of the cardiovascular lesions to immunologic factors required for their production. In an attempt to establish etiological responsibility for antigen, antibody, or interaction between the two prerequisite for the production of lesions, attention would be directed to questions regarding: (1) the type and quantity of streptococcal antigen, (2) the type, effective titer, and tissue deposition of the respective antibody produced, (3) the site of interaction between streptococcal antigen and antibody and the tissue response thus evoked.

The technique of passive sensitization and subsequent challenge with antigen again seemed to be the most satisfactory method in controlling and studying these variable factors. Since this method had been previously successful in studying horse serum-antihorse serum systems in the experimental production of arteritis in the rabbit[34] this technique was extended to similar investigation with streptococcal antigen-antibody systems. This report is concerned with the first of these to be studied, streptococcus toxin-antitoxin.

## MATERIALS AND METHODS

Streptococcus erythrogenic toxin* was prepared by growing *Streptococcus hemolyticus* N. Y. 5 strain on Veldee medium containing Difco bacto peptone and proteose peptone, sodium and calcium chlorides, magnesium sulfate, potassium phosphate. Dilutions were made in a solution of physiologic saline, 0.07 per cent potassium hydrogen phosphate, and 0.02 per cent human serum albumin as a stabilizer, at a pH 7.0. Potency of the final preparation used, as determined by the standard intradermal skin test in the rabbit, was 58,000 skin test doses per c.c.

Streptococcus anti (erythrogenic) toxin included two types: 1. Homologous; attempts were made to prepare an antitoxin in the rabbit by various schedules, doses, and routes of administration, including intravenous, intramuscular, and intracutaneous injections of streptococcus erythrogenic toxin alone and in combination with adjuvants. The animals were bled by cardiac puncture and the serum was tested for antitoxin titer by standard flocculation techniques[35, 36] and intracutaneous neutralization tests in the rabbit.[37] 2. Heterologous*; prepared from the serum of horses immunized with the streptococcus erythrogenic toxin described previously.

In the experimental procedure followed all experimental animals were albino rabbits of the New Zealand strain ranging in weight from 1.5 to 2 kilograms.

*Kindly supplied by and through the courtesy of Dr. H. A. Piersma, Director, Human Biological Section, Lederle Laboratories Division, American Cyanamid Company.

*Group I.*—Thirty rabbits were each given 1,500 U.S.P.H.S. units of sterile streptococcus antitoxin (horse globulin) intravenously. At periods varying between 2 to 6 hours later each animal received intravenously 5,000 S.T.D. sterile streptococcus erythrogenic toxin. Succeeding intravenous doses of toxin were given as follows: fifteen minutes later—10,000 S.T.D.; thirty minutes later—15,000 S.T.D. For purposes of administration the streptococcus erythrogenic toxin was diluted in physiologic saline (10,000 S.T.D. per milliliter), and all intravenous injections were given through the marginal ear vein.

*Group II.*—Five animals received only 1,500 U.S.P.H.S. units of the same lot of streptococcus antitoxin.

*Group III.*—Five animals received only 30,000 S.T.D. of the same lot of streptococcus toxin according to the same dose-time schedule as those animals in Group I.

Ten rabbits received 1,500 U.S.P.H.S. units of streptococcus antitoxin intravenously. Two to six hours later five of the animals received diphtheria toxin, 1/150 M.L.D. per 0.1 ml., intravenously *(Group IV)* and the five remaining received sterile Veldee medium intravenously *(Group V)*.

Schedule of administration of diphtheria toxin as follows: initial dose—0.5 ml., succeeding doses in fifteen minutes—1 ml.; thirty minutes later—1.5 ml. Veldee medium was diluted in physiologic saline so that the final volume was equivalent to the concentration of the Veldee medium found in the streptococcus toxin preparation. Equivalent doses of 0.5, 1, and 1.5 ml. were administered according to the same schedule used for doses of streptococcus toxin in Group I.

Five animals received 5 ml. normal horse serum intravenously *(Group VI)* and five received horse tetanus antitoxin 1,500 U.S.A. units intravenously *(Group VII)*. Two to six hours later all ten animals received streptococcus toxin intravenously according to the same dose-time schedule of Group I.

Twenty-four hours following the challenging doses of streptococcus erythrogenic toxin or substituted material used in the control experiments, the animals were sacrificed by air embolism. Sections were taken from the lungs, heart, kidneys, liver, spleen, pancreas, and adrenals. Hematoxylin and eosin stained sections of the tissues were studied microscopically.

## RESULTS

The serum of rabbits that had been injected with streptococcus erythrogenic toxin in attempts at immunization were found to contain either no demonstrable antitoxin or antitoxin of extremely low titer so as to render them impractical for experimental use in this procedure. When use of this antiserum was attempted in passive sensitization procedures followed by subsequent challenge with streptococcus toxin, pathologic lesions could not be demonstrated in any of the rabbits thus treated.

In preliminary experiments attempting to develop a satisfactory procedure for the production of vascular lesions by the method of passive immunization and subsequent challenge with specific antigen several different

techniques were employed. Variations were made in the relative doses of antitoxin and of toxin, routes of administration, the order in which each was injected, and the time interval between the administration of each. Only the method described in the previous section of this report resulted in the production of vascular lesions in some of the animals.

*Pathologic Findings.*—In Group I, fourteen of the thirty animals challenged with streptococcus erythrogenic toxin following passive sensitization with specific equine antitoxin, exhibited pathologic changes which were confined to the lung. The most constant finding was in the small pulmonary arteries and in the pulmonary arterioles. These showed chiefly perivascular and adventitial infiltration especially of eosinophils and, to a much lesser extent, with lymphocytes, plasma cells, and a few polymorphonuclear leukocytes (Fig. 1).

Eosinophils were occasionally found penetrating the media (Fig. 1, *A*, *C*, and *E*) and lying within the intima (Fig. 1, *A*, *C*, *D*, *E*, and *F*) of these vessels, and in a few instances eosinophils were found within their lumen. Minimal hyperplasia of the endothelial cells of the intima was occasionally seen (Fig. 1, *B*). Much less frequently observed was the occasional involvement of a pulmonary venule with perivenular cellular infiltration with accumulations of similar types of eosinophilic, lymphocytic, and neutrophilic cells. In these fourteen animals exhibiting a periarteritis occasionally a slight interstitial infiltration of eosinophils within the alveolar walls and submucosa of the bronchioles was seen. In grading the relative degrees of panarterial and periarterial cellular infiltration of these fourteen rabbits; two animals were considered to have severe arterial lesions (Fig. 1, *C*), four moderate, and eight mild.

The minimal incubation period between the injection of antitoxin and subsequent administration of toxin necessary for the production of lesions was found to be two hours. However, as the incubation period was increased and allowed to reach five to six hours, lesions were found in a greater percentage of animals and the individual vessels affected generally exhibited a more advanced degree of involvement.

In all of the animals of this group the other internal organs examined were microscopically normal.

No microscopic pathologic lesions were found in any of the thirty rabbits, of whom groups of five each had received intravenous injections of one of the following: streptococcus antitoxin only (Group II); streptococcus erythrogenic toxin only (Group III); streptococcus antitoxin plus diphtheria toxin (Group IV); streptococcus antitoxin plus Veldee media (Group V); normal horse serum plus streptococcus toxin (Group VI); tetanus antitoxin plus streptococcus toxin (Group VII).

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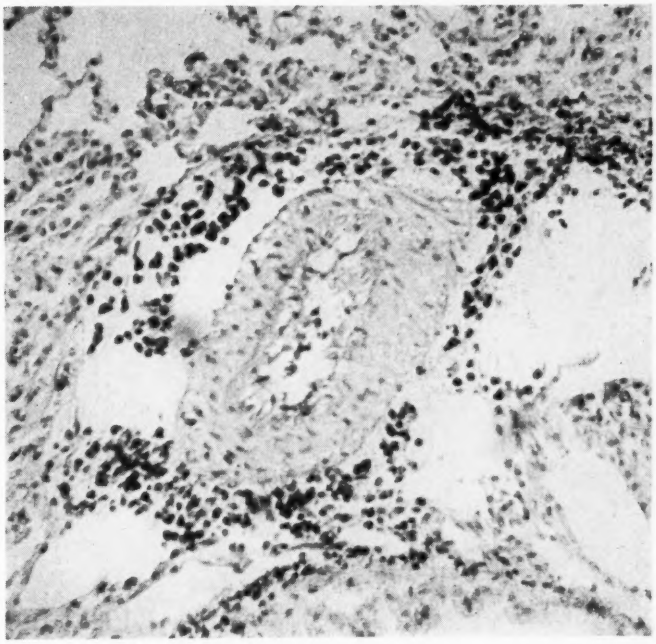
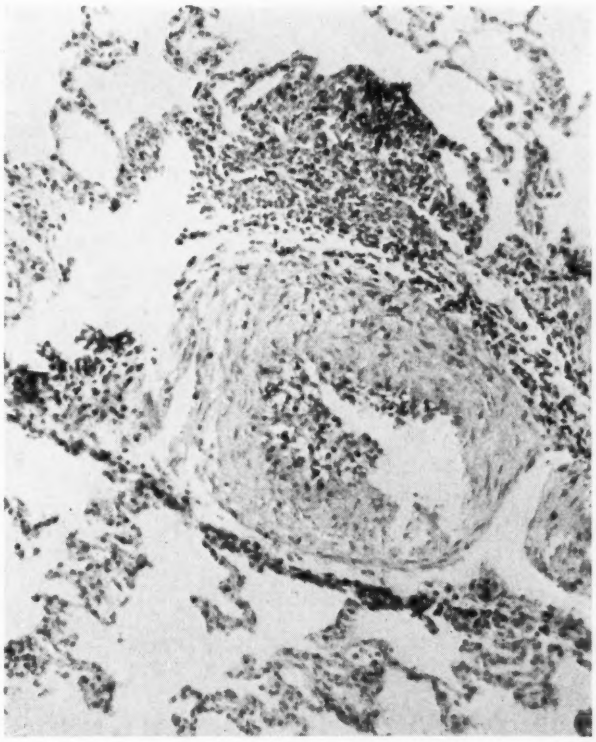
Fig. 1.—Sections of lungs from rabbits that had been passively sensitized with streptococcus antitoxin and subsequently challenged with streptococcus erythrogenic toxin. Small pulmonary arteries: *A* and *B*, cross section; *C*, tangential section of branching vessel; *D*, longitudinal section; *E* and *F*, pulmonary arterioles, cross sections. These demonstrate lesions of periarterial and adventitial infiltration with eosinophils, lymphocytes, some plasma cells and neutrophils. A few eosinophils are seen penetrating the media in *A*, *C*, and *E* and lying within the intima in *A*, *C*, *D*, *E*, and *F*. Intimal proliferation is seen in *B*. An unusually extensive degree of cellular infiltration is seen in *C*.

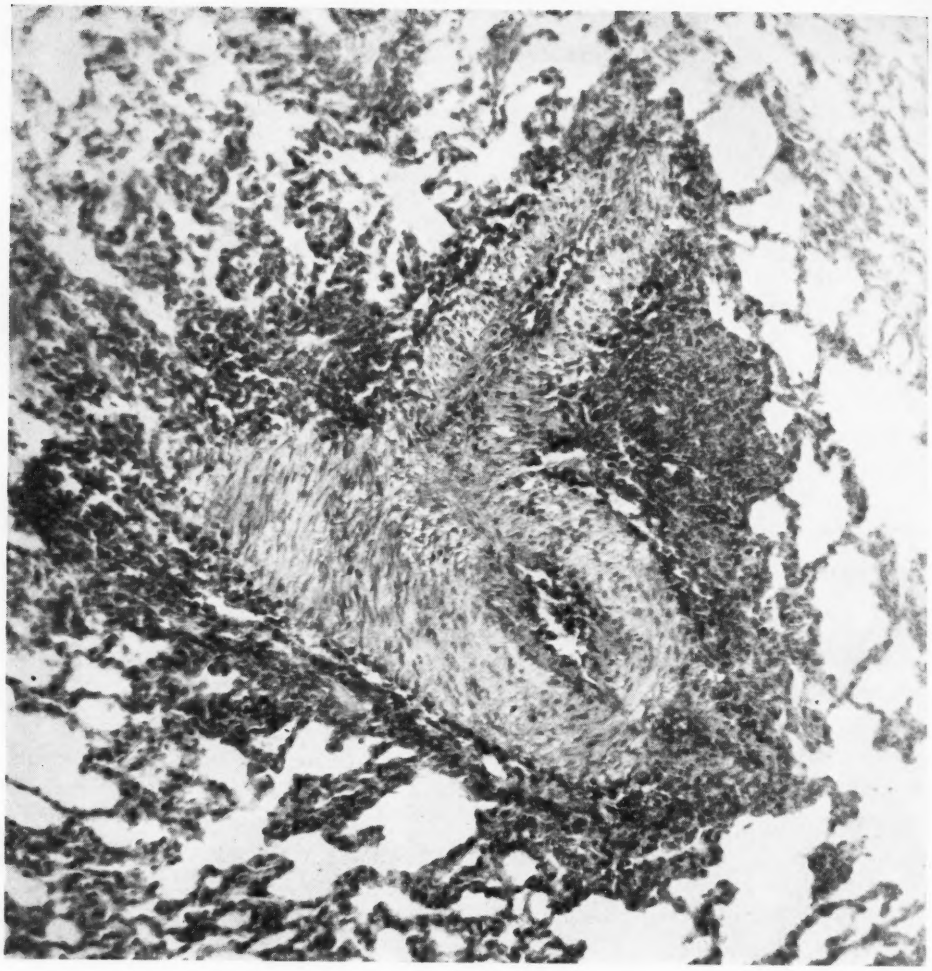


*A.*

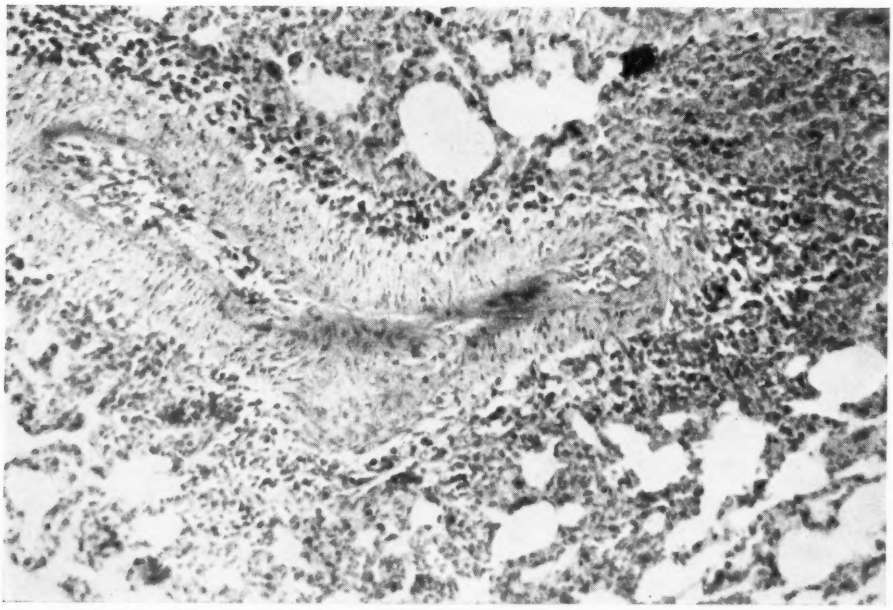


*B.*

Fig. 1*A* and *B*. (*For legend, see opposite page.*)

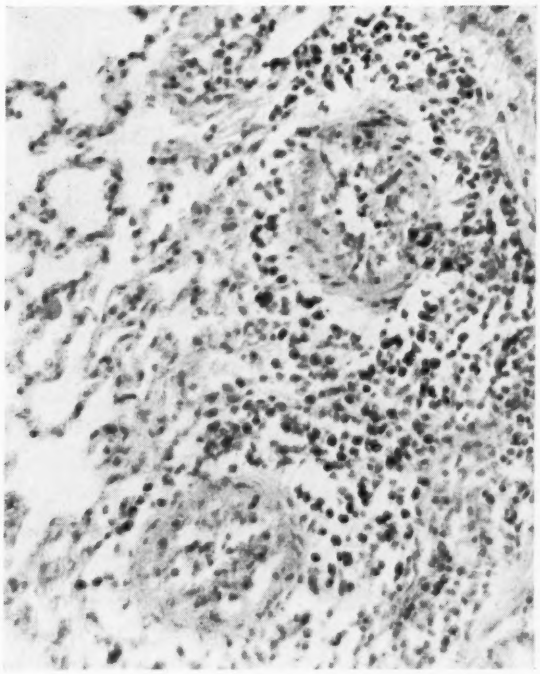


*C.*

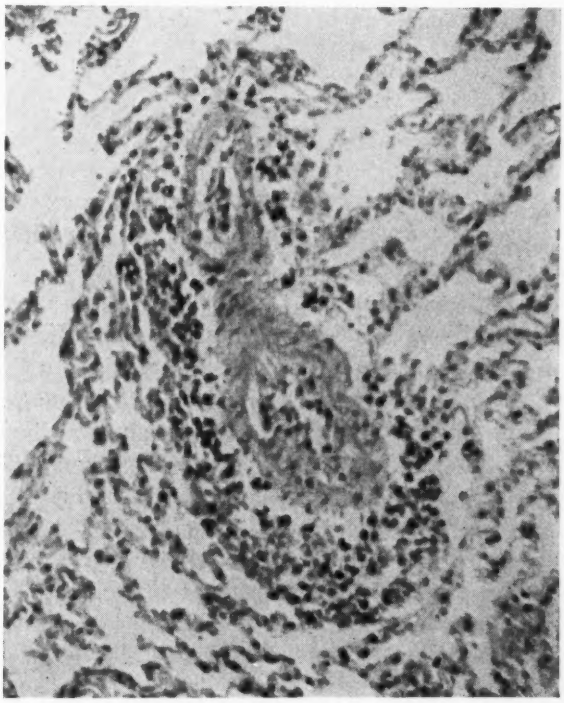


*D.*

Fig. 1*C* and *D*. (*For legend, see page 414.*)



*E.*



*F.*

Fig. 1*E* and *F.* (*For legend, see page 414.*)

## DISCUSSION

The object of this study as set forth was to determine whether streptococcus toxin, alone, apart from somatic cellular components of the streptococcus or its other extracellular products, could result in the production of vascular lesions similar to those found in experimental animals by using injections of the whole organism,[10, 21-25] or its cellular components,[11] or by inducing experimental streptococcal infections.[26, 28] Further, by employing the technique of passive sensitization with antibody (streptococcus antitoxin) and subsequent challenge with specific antigen (streptococcus toxin) variable factors such as the complete absence or presence of exact amounts of antigen and antibody and relative time relationships could be controlled with elimination of the nonspecific factors often associated with active sensitization. Thus, any pathologic vascular lesions resulting from the in vivo action of streptococcus toxin[12-20] might be more critically evaluated and further classified as to type arising as the direct result of: (1) the presence of streptococcus toxin, (2) sensitization to its specific antibody, or (3) the reaction of toxin plus antitoxin. With data of this type it was felt that the importance of the relative roles of toxicity and hypersensitivity could be more fairly assessed.

The first obstacle met was the inability to obtain a satisfactory rabbit streptococcus antitoxin. Our experience would thus tend to parallel that of Coffey[38] and Stock,[39] in finding that antitoxic serums cannot be prepared with satisfactory titer in the rabbit in contrast to its capacity for producing antibacterial serums of high titer. It is further noteworthy in this regard that pathologic cardiovascular or renal lesions were not found in those rabbits that received repeated injections of streptococcus toxin over a period of several weeks in attempts at immunization. Likewise, the use of these rabbit serums in passive sensitization-challenge techniques failed to result in the production of pulmonary vascular lesions in contrast to the results of similar procedures employing heterologous streptococcus antitoxin of high titer. One is then tempted to speculate that the reported vascular lesions produced in the rabbit by experimental streptococcal infections,[26, 27] if due to a hypersensitization mechanism, may be mediated through an antigenic fraction other than toxin produced in vivo. Also to be considered is the possibility that those lesions reported to result from the administration of streptococcus toxin,[12-14, 16-19] are not mediated through the production of a sensitizing antibody, and perhaps are due to a toxic action of the material used rather than an antigenic action of streptococcus toxin, per se. However, these do not necessarily follow as conclusions, for the possibility always exists that the rabbit may produce antitoxin of a type not detected by known serologic testing techniques or that the responsible sensitizing antibody is of the fixed tissue type. For it has been shown that rabbit antibodies of the skin sensitizing type appear to be quantitatively unrelated to antibody content as measured by precipitin titer.[40-42] Possibly other undetermined in vivo immunologic mechanisms not duplicated by our experimental procedures and techniques may come into play; for ex-

ample, the phenomenon of autosensitization which has been experimentally induced by an antigen resulting from the combination of bacteria or a bacterial toxin with normal tissue protein.[22, 43]

The original plan of the experiment was then altered and the procedure carried out employing heterologous streptococcus antitoxin of equine origin. Admittedly another nonspecific factor is introduced by employing heterologous species serum as the medium of transfer of the specific sensitizing or immunizing antibody to an otherwise normal rabbit. Likewise, this would not then necessarily represent the natural immunologic state in the animal during the course of a streptococcal infection. However, passive transfer of heterologous antiserum has been proved a valid procedure in sensitizing the rabbit to specific antibodies against bacterial fractions.[44] Furthermore, Dixon and his associates[45] have demonstrated that the rabbit handles heterologous species serum globulin much like its own serum globulins for a period of four days following its administration and the duration of this experiment of only twenty-four hours after the administration of horse antitoxin to the rabbit was well within this time limit. The specificity of the role of the antibody fraction is demonstrated in that the arterial lesions appeared in animals sensitized with streptococcus horse antitoxin plus streptococcus toxin and not when equivalent amounts of normal horse serum and streptococcus toxin were employed.

The lesions reported here, chiefly periarteritis and a mild panarteritis of the pulmonary vessels, produced only by the presence of streptococcus antitoxin plus streptococcus erythrogenic toxin, demonstrate a dependence upon a reaction of immunologic specificity. Negative findings in the groups of animals exposed to control factors ruled out a toxic action of the streptococcus toxin, a tissue reaction due to circulating or fixed antibody alone, and other etiological possibilities including streptococcus antitoxin plus an unrelated bacterial (diphtheria) toxin, the normal horse serum proteins plus streptococcus toxin, an unrelated (tetanus) antitoxin of equine origin plus streptococcus toxin, and streptococcus antitoxin plus the constituents of the media in which toxin was prepared.

This last point is of major importance especially since it is noted that streptococcus erythrogenic toxin is not a purified substance and has not been totally separated from other constituents of the media including proteose peptones and albumin stabilizer. Furthermore, lesions resulted only from the presence of the specific antitoxin plus streptococcus toxin in the animal under a set of standard conditions, namely, 1. the relative excess of antitoxin (1,500 units) over toxin (30,000 S.T.D.). This represents a ratio of antibody two and one-half parts to antigen one part since 1 U.S.P.H.S. unit of antitoxin neutralizes 50 S.T.D. of toxin. The reason for this required ratio is not apparent. Perhaps it is related to the combining powers of antigen and antibody or perhaps it insures the retention within the body of the minimum required amount of available antibody following its tissue distribution or partial elimination as a foreign protein. 2. Allowing an incubation period of five to six hours after the administration of antitoxin before toxin is injected. Pre-

TABLE I*

| TECHNIQUE | ANTIHORSE SERUM-HORSE SERUM | STREPTOCOCCUS ANTITOXIN-ERYTHROGENIC TOXIN |
|---|---|---|
| Sensitizing injection | Homologous antiserum | Heterologous antiserum |
| Optimum incubation period prior to challenging injection | ½ hr. | 5 to 6 hr. |
| Type of pathologic lesion found | Periarteritis<br>Panarteritis | Periarteritis (chiefly)<br>Panarteritis (mild) |
| Location of lesion | Small pulmonary arteries and arterioles | Small pulmonary arteries and arterioles |
| No. of animals showing lesions | 12/12<br>(100%) | 14/30<br>(47%) |
| Average degree of severity of lesion | +++ | +-++ |

*Summary of similarities and differences in techniques required and results obtained in the experimental production of arteritis by passive sensitization and challenge in the rabbit, using (1) antihorse serum-horse serum, and (2) streptococcus antitoxin-streptococcus erythrogenic toxin systems.

sumably this represents the time required for the fixation of antibody to the shock tissue. 3. Injecting antitoxin into the blood stream before the intravenous administration of toxin. This may possibly indicate that for the production of an arterial lesion it is necessary that the antibody be fixed while reacting with circulating antigen. It is only a matter of speculation, but perhaps this may be one of the conditions that distinguishes a sensitizing action within the vessel wall from a pure neutralizing action within the lumen of the vessel as an outcome of the union of antigen and antibody in vivo.

In comparing the technique and results in this study with those in which similar lesions of arteritis were produced with the identical technique of passive transfer and challenge utilizing antihorse serum-horse serum systems[34] certain differences and similarities were noted. These are summarized in Table I. Differences are noted in the species antiserum used, the incubation periods required, and the uniformity and severity of the lesions produced. But there were marked similarities in tissue distribution and histologe appearance of these lesions. Any reasons for these findings are not apparent. Perhaps these differences may be related to inherent biologic or biochemical differences in homologous and heterologous antiserums, tissue responses to these, or perhaps due to immunochemical differences residing within the different antigen or antibody molecules themselves, for it has been shown that antibody molecules produced against two different type antigens even in the same animal possess different physicochemical properties.[46] Regarding site of involvement, Hawn and Janeway[47] and Ehrich, Seifter, and Forman[48] have reported that lesions produced in different organs in rabbits depended upon the particular protein fraction of the foreign serum injected, and Pressman, Sherman, and Korngold[49] have demonstrated by tracer studies that certain serum proteins have a preferential organ site of localization. Possibly in this instance it may be dependent upon the fact that the pulmonary arteriole is considered the "shock organ" in the rabbit, or perhaps is related to the intravenous route of

administration of the challenging antigen. This later factor might explain why pulmonary lesions predominated over, for example, renal lesions in that the toxin first contacted the pulmonary vasculature and was removed by or neutralized in the lungs before reaching the renal arteries.

However, this reasoning is not entirely valid in that there were no cardiac lesions, and antigen and antibody when introduced intravenously should course through the right heart before circulating through pulmonary vessels; unless, of course, the presence of cardiac lesions is dependent upon antigen and antibody reaching the myocardium via coronary arteries. Regarding appearance, the pulmonary vascular lesions of periarteritis and panarteritis in both antihorse serum-horse serum and streptococcus antitoxin-toxin groups, were identical in histologic detail. The lesions observed in the streptococcus antitoxin-toxin sensitized group of rabbits occurred much less frequently (50 per cent of animals) than in the antihorse serum-horse serum group (100 per cent of animals) and generally were of a milder nature and represented a less severe degree of tissue damage. However, apart from quantitative differences there were no qualitative differences and it is possible that lesions in both groups represented tissue manifestations of subacute anaphylaxis. At any rate there was nothing distinctive about the tissue response to sensitization with streptococcal antitoxin-toxin that set it apart from sensitization to a foreign protein system such as antihorse serum-horse serum.

The reason for the failure to produce endocardial, myocardial, renal lesions, arterial lesions in other internal organs, and fibrinoid degeneration of collagen in the pulmonary arterial lesions, as reported by other investigators[10, 28] likewise is not evident. Ehrich[29] has attempted to relate the histologic characteristics of the vascular lesion to either antibody production or a response to nonspecific tissue damage. More and Kobernick[23] feel that perhaps necrosis of the muscle cells of the media may be a later stage in the evolution of experimentally induced arterial lesions in the rabbit as similarly pointed out by Duff[50] regarding lesions of periarteritis nodosa in man. Perhaps if longer than twenty-four hours had been allowed for the evolution of the arterial lesions other changes, including fibrinoid degeneration of collagen and necrosis of the media might have been observed or perhaps the lesions might have resolved. However, in this experimental procedure it was not considered practical to allow longer than twenty-four hours to elapse following passive sensitization with heterologous antiserum, for then a histologic study of the internal organs might be complicated by the factor of tissue response as a manifestation of antibody production to horse serum proteins.

In no way is it implied that sensitization to streptococcus erythrogenic toxin does not result in arterial lesions characterized by fibrinoid necrosis of collagen or other lesions of the vascular system, heart, kidney, or other organs. Simply, it has been impossible to corroborate this possibility by the technique used here. The question thus raised can be answered only by further experimental data. Matters for further investigation also include application of the technique of passive sensitization to a study of other streptococcal antigen-antibody systems including somatic antigens (group specific C carbohydrate

and type specific M protein) and other extracellular antigenic components (for example, Streptolysins O and S, fibrinolysin, hyaluronidase, proteinase, desoxyrinoneuclease, and riboneuclease).

## SUMMARY

1. Thirty rabbits were passively immunized with 1,500 U.S.P.H.S. units of streptococcus antitoxin (horse globulin) and beginning two to six hours later were given 15,000 S.T.D. streptococcus erythrogenic toxin in divided doses.

2. Twenty-four hours later these animals were sacrificed and sections taken for microscopic study. Microscopic pathologic lesions were found in fourteen of the rabbits and involved the small pulmonary arteries, pulmonary arterioles, and occasionally the pulmonary venules. The lesions consisted chiefly of a periarteriolar and a mild panarteriolar infiltration especially of eosinophils and to a lesser extent of lymphocytes, plasma cells, and neutrophils.

3. Five rabbits received a similar dose of streptococcus antitoxin alone, and five received the same dose of streptococcus toxin alone. Ten rabbits were passively immunized with streptococcus antitoxin and of these, five were challenged with diphtheria toxin and the remaining five with Veldee media. Five rabbits were passively immunized with tetanus antitoxin and five received normal horse serum prior to the administration of challenging doses of streptococcus erythrogenic toxin. In all thirty of these animals there were no pulmonary vascular lesions observed after twenty-four hours.

4. The lesions of pulmonary periarteritis and panarteritis in those rabbits passively immunized against and challenged with streptococcus erythrogenic toxin were less frequently and uniformly produced and were of a less severe degree of involvement than found in a series of rabbits that had been passively sensitized against and challenged with horse serum.

5. Apart from quantitative differences, qualitatively the lesions were histologically identical in rabbits subjected to passive sensitization and challenge, regardless of whether the antibody-antigen system employed was homologous antihorse serum-horse serum or heterologous species streptococcus antitoxin-erythrogenic toxin.

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# RAGWEED OIL DERMATITIS

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RAGWEED oil dermatitis was first described by Hannah[1] in 1919 and again reported by Sutton[2] in the same year. This is almost always a seasonal dermatitis, occurring for the most part from early August to the first killing frost. It has, however, other features which complicate the dermatitis and it is therefore often misdiagnosed or overlooked. In the last ten years we have collected over 25 cases of this dermatitis. Since there has been a paucity in the allergic literature of reports from the time of the first excellent survey by Brunsting and Anderson,[3] it was believed that a review of the subject would be helpful to dermatologists and allergists, as well as to general practitioners who are usually the first to see this condition.

In 1931, Brown and associates[4] showed that it was the oily fraction of the ragweed pollen which was the sensitizing agent. The ragweed pollen has two chief components: an inner core which contains the water-soluble protein fraction used by allergists in the treatment of hay fever and an outer portion which is oil-soluble and comes in intimate contact with the sensitized individual at the time of ragweed pollination. Subsequently we have learned that patients who contact the stem or the leaves of the ragweed plant will also suffer from the dermatitis, but the distribution in these cases is limited to the area of contact, rather than the diffuse or disseminated distribution usually observed in air-borne involvement.

## DESCRIPTION OF THE DERMATITIS

Ragweed oil dermatitis is considered to be an air-borne pollen dermatitis. It therefore has a distribution on the skin at points of contact and these are especially the eyelids and periorbital areas, the sides of the face, the sides of the neck, and the exposed areas on the arms. We have never seen a patient with this dermatitis who did not have involvement of the eyelids. Many other contact agents will regularly produce this type of dermatitis, and it is especially important to recognize the seasonal nature of this disease and to differentiate it from industrial contact dermatitis and also from other forms of nonindustrial contact dermatitis. Furthermore, it must be differentiated from atopic dermatitis, especially the atopic dermatitis with seasonal occurrence, probably due to the ragweed pollen protein. At times the distribution may suggest seborrheic factors since in milder cases there may be a scaling of the scalp, mild lichenification and scaling of the eyelids with a similar appearance around the nasal folds. Ragweed oil dermatitis descends to the V of the neck and sternal area, as

Presented before the Ninth Annual Meeting of the American Academy of Allergy, Boston, Mass., February, 1953.

Received for publication March 14, 1953.

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a rule, and hence it may be confused with seborrheic dermatitis in its milder forms. It is of interest that on the upper extremities the distribution is almost always on the extensor surfaces, whereas in atopic dermatitis the flexor areas are regularly involved.

Occasionally a distribution of the dermatitis may be seen which involves the sites of direct contact with the antigen whether it be ragweed pollen, stem or leaf. A farmer who handles winter fodder contaminated with ragweed plant or pollen will show more involvement of the dorsum of the hands and forearms. There is less involvement of the face, eyelids, and sides of the neck.

There is a certain similarity between the course of ragweed oil dermatitis and ragweed hay fever. The first year of involvement, the patient may report only minimal dermatitis which promptly subsides when the plant and pollen disappear following the first killing frost. As the untreated disease continues, however, the dermatitis comes earlier and stays later. Furthermore, in long-standing disease other sensitizations occur which complicate the picture. In hay fever, likewise, it is not unusual for long-continued and untreated disease to be followed by chronic vasomotor rhinitis and bronchial asthma. Patients commonly give the story of seasonal asthma which promptly subsides with the frost, but as the years go by the asthma persists into the Thanksgiving, then the Christmas period, and finally becomes perennial. So with ragweed dermatitis we have seen patients who have perennial dermatitis and have by that time developed a number of other sensitivities unrelated to the initial ragweed oil dermatitis.

Other observers have noted and reported this multiple sensitivity in patients with chronic ragweed oil dermatitis. We have noted one finding, not previously described, which is the relationship between ragweed oil dermatitis and sensitivity to ultraviolet light in some patients. It is of interest that not only does the ragweed oil contact the uncovered skin surfaces (face, neck, and arms), as noted previously, but also this is the area most commonly exposed to the sun. In this connection, actinic exposure in subacute lupus erythematosus of the discoid variety not infrequently has a distribution on the face, V of the neck, and extensor surfaces of the arms, and offers a further condition to be differentiated from ragweed oil dermatitis. This particular feature of photosensitivity or phototoxicity was brought to our attention by the following case.

CASE 1.—The patient, a woman aged 50, was first seen at the outpatient department of the Massachusetts General Hospital on Aug. 27, 1946. At that time she reported a seasonal dermatitis of twelve years' duration. The basic nature of her difficulty was recognized in both the allergy and dermatology departments, and following a series of patch tests a bullous reaction to ragweed oil was noted. At that time, too, she showed a consistently positive reaction to timothy oil and negative patch tests to thirty other commonly used agents in industry and in the household. For two years she was given bland washes and lotions. Beginning in 1948 she was treated at regular intervals with increasing doses of ragweed oil by injection, and for several seasons she showed 70 per cent progressive improvement in the dermatitis. In the 1952 season, however, it was noted that particularly during the dry days of July and early August, the dermatitis had flared out of proportion to the expected seasonal flare. Topical therapy was of no avail and the patient at this time presented a superficial, erythematous, scaly dermatitis, without frank vesiculation, on the lids, periorbital areas,

sides of the face, and the extensors of the arms. She had been using diluted Burow's solution and a bland paste of the water emulsion type, and during the day she used a soothing lotion. It was finally decided to prescribe a sun-screening agent, para-aminobenzoic acid, in a water-soluble base. The response to this therapy was dramatic and trials with similar base without the para-aminobenzoic acid resulted in a flare of the dermatitis.

## PATCH TESTS

In the investigation of these patients (Table I), the history and physical examination to include the chief features previously mentioned usually suffice to establish the diagnosis. All patients in this series showed a positive patch test to ragweed oil. Standards for patch testing have been established for thousands of antigens. There is relatively little information available for patch testing with ragweed oil. Oleoresins of plant oils have been available for many years, but often the material undergoes changes with time and false reactions may occur. With care and particularly with refrigeration these extracts are reasonably reliable. Patch testing with fresh material (grasses and weeds) can be done. This is helpful in the accuracy of one phase of the testing of the patient, and enables the investigator to test with a wide variety of materials which are indigenous to the patient's environment. It must be remembered that some plants are primary irritants by virtue of their physical characteristics, for example, roughness or horny spines. Indiscriminate patch testing with ragweed oil can lead to focal and general flare of the eruption, depending on the sensitivity of the patient, the strength of the extract, and the amount of cutaneous involvement at the time of patch testing.

CASE 2.—The patient, a man aged 65, was first seen in December, 1935, at the Massachusetts General Hospital on the dermatologic service. He reported a seasonal dermatitis on the exposed areas of the face, neck, and extensors of the arms, of ten years' duration. The patient at that time was a guide in the Maine woods. This area is not ragweed-free. The dermatitis steadily grew worse and after ten years there was always some evidence of subacute dermatitis to be seen. The 1935 seasonal flare was worse than any previous recurrence, and he sought hospital care in December, 1935. A diagnosis of ragweed oil dermatitis was made by the attending dermatologist. The allergist who made the patch tests quoted the work of Brown and associates in his note in the record. No injection therapy was available. There was a remission following topical therapy and the patient was again admitted in December, 1936, with a recurrence. Thereafter he changed his locale and his work and became a gardener in the Boston area. He worked (in part) for a dermatologist and was given a few injections of ragweed oil extract on a phylactic basis. In January, 1950, he again appeared in the allergy outpatient department at the request of his dermatologist. A patch test to ragweed oil (1 to 100 dilution) was applied. Within twelve hours the patient showed a bullous reaction at the site of the test and a generalized flare of all sites previously involved during the ragweed season. He was admitted to the hospital for a week of symptomatic treatment and the patch test remained positive for seven weeks. Subsequently he was placed on a desensitizing regimen, with excellent results.

The persistence of the positive patch test with some antigens, particularly ragweed oil, is, to some extent, a clue to the chronicity of the clinical eruption. Rhus dermatitis is usually a self-limited eruption with a variable period of involvement persisting for two to four weeks. Likewise, a positive patch test to rhus toxicodendron usually disappears in five to ten days. It would seem that the ragweed antigen makes a firmer bond with the protein of the skin than

TABLE I. SUMMARY OF CASES

| CASE | AGE AND SEX | OCCUPATION | DATE FIRST SEEN | DURATION OF SYMPTOMS | RESPIRATORY ALLERGY | OTHER CONTACT FACTORS | PATCH TESTS | INTRADERMAL TESTS | TREATMENT |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 65 M. | Railroad yard worker | November, 1952 | 15 yr. | Asthma | None | Ragweed oil 4+; other patch tests negative | Inhalant tests positive | Symptomatic |
| 2 | 70 M. | Horse trainer | October, 1951 | 3 yr. | None | None | Positive to ragweed oil | None | Ragweed oil injections 1951 |
| 3 | 57 M. | Telephone linesman | September, 1952 | 8 yr. | None | None | Positive to ragweed oil; routine patch tests negative | Negative | Ragweed oil injections 1952-1953 |
| 4 | 58 M. | Building superintendent | October, 1951 | 8 yr. | None | None | Ragweed oil, other patches negative | None | Ragweed oil injections 1951-1953 |
| 5 | 76 M. | Shoemaker and gardener | October, 1940 | 13 yr. | None | None | Ragweed oil positive; other patch tests including leathers and dyes negative | — | None |
| 6 | 70 M. | Farmer | October, 1948 | 5 yr. | None | None | Ragweed oil positive | None | Symptomatic only |
| 7 | 36 M. | Farmer | October, 1941 | 12 yr. | None | None | Ragweed oil positive | None | No symptoms in Europe in military service |
| 8 | 40 M. | Laborer (rural) | December, 1950 | 14 yr. | None | None | Ragweed oil positive | None | No symptoms when out of country |
| 9 | 62 M. | Mailman | — | 25 yr. | None | None | Ragweed oil positive | None | Ragweed oil injections |
| 10 | 84 M. | Retired, rural area | November, 1949 | 34 yr. | None | None | Ragweed oil positive; edema 48 hr.; vesicles 96 hr. | None | — |
| 11 | 56 M. | Laborer | October, 1949 | 12 yr. | None | None | Ragweed oil positive | None | — |
| 12 | 65 M. | Shoe manufacturer | 1948 | 9 yr. | None | None | Ragweed oil positive; timothy positive; red top positive | None | Ragweed oil injections 1949-1953 |
| 13 | 45 M. | Car dealer | 1946 | 9 yr. | None | None | Ragweed oil negative | — | Ragweed oil injections 1948-1953 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 14 | 58 F. | Housewife | August, 1934 | — | Hay fever in childhood, atopic dermatitis | None | Ragweed oil 4+ (for general flare) | Positive to ragweed and other proteins | Injections since 1949 |
| 15 | 62 M. | Carpenter | October, 1945 | 7 yr. | None | None | Ragweed oil 3+; ragweed pollen 2+; bullous reaction; 40 patch tests negative | Negative | — |
| 16 | 36 F. | Waitress | July, 1945 | 10 yr. | None | None | Ragweed oil 4+; retested 5 yr. later, still positive | Scratch tests negative | — |
| 17 | 67 F. | Retired, rural area | Sept. 27, 1948 | 24 yr. | None | None | Ragweed oil 3+; pyrethrum negative | Scratch tests negative | Injections since 1948 |
| 18 | 50 F. | Housewife | August, 1946 | 17 yr. | None | None | Ragweed oil 4+; repeated 3 times; timothy oil 3+; 30 patch tests negative | — | Injections since 1948 |
| 19 | 47 M. | Painter | October, 1951 | 5 yr. | None | None | Ragweed oil 4+ | — | Injections since 1951 |
| 20 | 51 M. | Welder, carpenter | September, 1946 | 7 yr. | None | None | Ragweed oil 4+; timothy oil negative | Skin tests, scratch tests negative | Injections since 1946 |
| 21 | 52 F. | Laundress | 1944 | 8 yr. | Nasal polyps | None | Ragweed pollen 2+; ragweed oil 2+; other tests negative | Scratch tests negative | Injections since 1949 |
| 22 | 77 F. | Housewife | September, 1950 | 25 yr. | None | None | Ragweed oil 4+; timothy oil 2+; 8 grasses and pyrethrum negative | Scratch tests negative | Injections since 1951 |
| 23 | 64 F. | Worked outdoors in the Navy yard | May, 1945 | 8 yr. | Hay fever 24 yr., ragweed type | ? of blueprints | Ragweed oil 2+; birch oil 2+; all vegetable oils negative; grapefruit peel 2+ | Scratch tests positive to ragweed protein | Began in 1947 with oil and protein fractions |
| 24 | 65 M. | Farmer, gardener and guide (woodsman) | December, 1935 | 27 yr. | None | None | Pyrethrum 3+; ragweed oil 3+; tansy 2+; metals 0 | Scratch tests negative | Injections since 1950 |
| 25 | 64 F. | Housewife | 1946 | 26 yr. | None | None | Ragweed oil 2+ | Scratch tests negative | Injections since 1946 |

do other antigens. Subsequent to our experience with this patient, we have never initially patch tested a patient suspected of sensitivity to ragweed oil with a higher concentration than a 1 to 1,000 extract. This strength has been sufficient for diagnosis in most cases. When necessary, dilutions of ragweed oil, 1 to 100 and 1 to 10, may be used. In our opinion, ragweed oil is not a primary irritant. A review of 100 records of contact dermatitis in which ragweed oil was used as one of the investigating patch tests did not yield a single positive reaction to ragweed oil.*

In some regions of the United States and Canada the general problem of weed oil dermatitis requires more extensive patch testing. Certain areas support a multitude of oil-bearing weeds causing dermatitis, and the exposure of individuals is greater incident to their occupation. The great farm belt areas report an increased number of patients with weed oil dermatitis. On the west coast the incidence is much reduced and is considered practically negligible. An appreciable number of patients with weed oil sensitivity exist along the eastern seaboard, and the frequency of involvement increases as one moves westward through the Middle Atlantic States to the Midwest. On the east coast, ragweed is the most common offender. Likewise, in the Minneapolis area, short ragweed is still important, but Mackoff and Dahl[5] found it necessary to look for other plant oil sensitivities. This was noted in their detailed report of antigenic sensitizing relationships between botanical tribes. Differences in antigenic properties were reported within members of the same family.

Finally, in any consideration of patch testing, it must be remembered that the patient may have additional or associated sensitivities. Pyrethrum has been incriminated more often than any other antigen in our series. Other environmental antigens must be carefully considered in a survey of the patient's contacts to include his industrial, home, hobby, and recreational contacts. These include sensitizers as well as substances known to be primary irritants. Gardeners and farmers, for example, are exposed to complex chemicals in fertilizers, insecticides, and detergents. Brunsting and Anderson[3] noted a number of positive patch tests in his series to pyrethrum, turpentine, and burweed marsh elder. Jordan and associates,[6] in their ragweed oil series, also reported sensitization to feverfew, bitterweed, flax, and linseed oil. It is obvious, therefore, that the investigational approach to ragweed oil dermatitis in some patients must be carried out with due consideration for the patient's total contact environment.

## TREATMENT

Although this paper is primarily presented for the purpose of diagnosis of ragweed oil dermatitis, it seems in order to record our observations of the treatment of this disorder. Brunsting and Anderson[3] reported discouraging results with desensitization procedures using ragweed oil in increasing doses by injection. Slater[7] reported relief of symptoms by oral desensitization in 2 patients. Shelmire,[8] in 1939, and Sheldon and Blumenthal,[9] in 1941, reported

*Material previously investigated by Dr. E. A. Polcari, Cambridge, Mass.

favorable results in desensitization measures using oral extract in increasing dosage. Fisher[10] reported a series of 18 patients with a five-year follow-up of 4 patients who were successfully treated by oral extract.

The patients in this series were treated by subcutaneous injection of increasing doses of a 10 per cent ragweed oil antigen dissolved in olive oil. Most patients were started on a 1 to 1,000 dilution and subsequently were able to tolerate injections from the 1 to 10 dilution. Patients received varying degrees of relief of symptoms, comparable to the injection therapy of hay fever. All patients beginning treatment were urged to follow a perennial form of therapy. Results improved with continued treatment. A combination of ragweed protein and ragweed oil was found useful in one patient. Patients were treated symptomatically if they came in during or immediately after the ragweed season, and injections were omitted except in treated cases already on a perennial basis. In this latter group the dosage during the season was reduced. Reactions of the focal and general type may be seen. General reactions usually involve sites of previous eczematous localization.

## DISCUSSION

It is evident from Table I that ragweed oil dermatitis is a specifically acquired epidermal sensitization, affecting most commonly the man of over 40 who is an outdoor worker. It is seasonal in occurrence, at first usually lasting from August to frost, but as the patient advances in age and as the seasonal exposures continue, the season may begin in June and extend to December. The localization in the early uncomplicated case is usually the face, including the eyelids, the sides of the neck, and the sternal area, as well as the extensors of the arms, sharply limited by the protective undershirt. In more chronic cases the dermatitis extends to involve the trunk, groin, and lower extremities, and may become generalized and take on the character of an exfoliative dermatitis. In our experience there have been no spontaneous permanent remissions in ragweed oil dermatitis. The longest duration has been twenty-eight years, in the patient who was a guide in the Maine woods. Although the dermatitis recurs every year, the untreated patient often reports variations in severity, which is similar to the experience of the untreated hay fever patient. It must be emphasized that the majority of these patients do not have atopic dermatitis and have no evidence of asthma or hay fever. Only 2 cases in this series were atopic and, in addition to ragweed oil, these patients have been treated with small intracutaneous doses of ragweed pollen protein extract.

Whereas a multitude of contact dermatitis problems remain insoluble as to their causal agent, these cases lend themselves to diagnosis from the history of a seasonal dermatitis and a great suspicion that one is dealing with an airborne pollen or plant contact or both. The diagnosis is verified by patch testing during a period of remission with a suitable extract of ragweed oil. The closed method of testing seems more suitable in our experience, although positive tests may be obtained with the open method using acetone preparations of the oil. Of additional interest was the five-day delay in the appearance of a positive patch test in a patient on ACTH gel therapy. This patient was a 60-year-old

railroad worker in rural New York State who had a seasonal dermatitis for many years undiagnosed, and who eventually suffered an exfoliative dermatitis following the 1952 ragweed season. Two weeks of hospitalization with topical and ACTH therapy resulted in a dramatic remission. Patch tests with various antigens including ragweed oil, 1 to 10 in olive oil, were negative at the forty-eight hour period. Three days later the patient reported itching at the site of the ragweed oil test which then became maximal in thirty-six hours and was followed by a typical eczematous reaction. Generalized flare was prevented by the use of ACTH gel and antihistaminics.

Although most observers have favored the oral route in the specific treatment with ragweed oil, the patients in this series were treated with ragweed oil by injection. All patients tolerated an initial dose of 1 to 1,000 by subcutaneous injection. Increments of 0.1 to 0.2 c.c. were used to reach a maintenance dosage of the 1 to 10 dilution in most instances. The intracutaneous method of treatment was not used. Reduction in epidermal sensitization could not regularly be demonstrated in this series even though patients showed good clinical protection. The percentage of relief ran parallel to results commonly seen in hay fever treatment. Perennial treatment was urged for all patients and the percentage of improvement increased with the length of treatment. This agrees with the experience of other workers in this field and also agrees with observations made in patients who are undergoing poison ivy "desensitization." Patients who do not improve with specific ragweed oil therapy should be investigated particularly for other plant-oil sensitizations. Additional industrial or environmental contacts with sensitizers or primary irritants must be considered. Finally, we must consider that photosensitivity or phototoxicity may play an important role in the continuation of the dermatitis in some patients. A number of our patients demonstrated a troublesome dermographism after the initial dermatitis, which required symptomatic treatment, had subsided.

## CONCLUSIONS

The diagnosis and clinical course of patients with ragweed oil dermatitis are presented. The chief features of the differential diagnosis are discussed. This type of dermatitis which rarely undergoes spontaneous remission must be differentiated from other forms of contact dermatitis due to industrial or environmental contacts and from seborrheic dermatitis, atopic dermatitis, actinic dermatitis, and lupus erythematosus. This specifically acquired epidermal sensitization occurred in only 2 patients who had asthma or hay fever.

Patients with ragweed oil dermatitis are often disabled and the eruption may be protracted. Generalization of the eruption was seen in 20 per cent of the patients studied. A positive patch test may be obtained in 100 per cent of patients with ragweed oil, using pollen, stem, or leaf as testing material. The general problem of patch testing in this group of patients and the pitfalls and complications are discussed. The allergist can call on his knowledge of botanical relationships of weeds to assist in the diagnosis. Photosensitization was observed in two (8 per cent) of the patients.

Treatment involves avoidance of exposure or specific desensitization or both, either by the oral or injection route of the ragweed oil antigen. Injection therapy follows the general principles used in routine preseasonal and perennial pollen (protein) therapy. Symptomatic topical therapy is helpful. Corticotrophic preparations are reserved for patients in the hospital with generalized dermatitis. Injection therapy with ragweed oil gives results comparable to those seen in routine hay fever "desensitization." The allergist alert to the problem of ragweed oil dermatitis is admirably equipped to manage successfully this not infrequent condition.

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# USE OF SOYBEAN MILK AS AN AID IN PROPHYLAXIS OF ALLERGIC DISEASE IN CHILDREN

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THE purpose of this study was to determine whether the incidence of atopic dermatitis (eczema) and other major allergies, as well as their allergic sequelae in potentially allergic infants might be influenced by completely withholding cow's milk from their diets from birth until the age of six to nine months. By a potentially allergic infant is meant an infant who has one or more allergic parents or siblings. A previous publication[1] has discussed the underlying reasoning for, and demonstrated the feasibility of, raising infants in this manner.

Ninety-one infants comprised the experimental group. They were all either offsprings or siblings of one or more individuals with one or more major allergies, that is, asthma, pollinosis (rose or hay fever), atopic dermatitis (eczema), or perennial allergic rhinitis (P.A.R.). Cow's milk was deliberately withheld from each infant from birth. Each was fed a commercially available soybean formula.* Table I indicates the age at which these infants were changed from soybean milk to cow's milk. Table II shows the length of the follow-up of this group which was continued until the time of this writing.

The histories of sixty-five siblings of those in the experimental group were also reviewed. In addition, a second control group was chosen as follows: From reviewing the histories of 4,710 allergic individuals in 1,215 families, 175 children with approximately the same allergic family history were selected. Those in the sibling group, as well as those in the nonrelated control group, were considered as suffering from a major allergy *only* if their allergic symptoms occurred *by or before* the age of onset of their counterparts in the experimental group. For each group the following data were sought: (1) the total incidence of major allergic diseases, (2) the incidence of atopic dermatitis, (3) the incidence of other allergies to cow's milk (colic, diarrhea, etc.), and (4) the allergic sequelae of atopic dermatitis and other allergies due to cow's milk.

Table III summarizes the comparative data obtained from the three groups of infants studied. Major allergy occurred in 14 per cent of the experimental group, in 64 per cent of the sibling controls, and in 52 per cent of the unrelated control group. The chances that these findings are due merely to coincidence is less than one in one hundred (Chi square equals sixteen). The differences in

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Presented Feb. 26, 1953, at the Ninth Annual Meeting of the American Academy of Allergy, Boston, Mass.

This work was done as part of the requirements of the Fellowship in Pediatric Allergy held by Dr. Johnstone.

Received for publication, May 26, 1953.

*Mull-soy, manufactured by the Borden Company.

TABLE I. AGE WHEN COW'S MILK INTRODUCED IN DIET OF EXPERIMENTAL GROUP—91 INFANTS

| AGE | NO. OF PATIENTS |
|---|---|
| Under 1 mo. | 5 |
| 1-3 mo. | 19 |
| 3-6 mo. | 17 |
| 6-9 mo. | 46 |
| Over 9 mo. | 4 |

TABLE II. LENGTH OF FOLLOW-UP OF EXPERIMENTAL GROUP—91 INFANTS

| FOLLOW-UP | NO. OF PATIENTS |
|---|---|
| 7-12 mo. | 6 |
| 1-2 yr. | 18 |
| 2-5 yr. | 43 |
| 5-11 yr. | 24 |

TABLE III

| | EXPERIMENTAL GROUP FED SOYBEAN MILK FROM BIRTH | SIBLINGS | NONRELATED CONTROL GROUP |
|---|---|---|---|
| Incidence of major allergy | 14/91 (14%) | 41/65 (64%) | 91/175 (52%) |
| Incidence of atopic dermatitis | 7/91 (8%) | 21/65 (32%) | 50/175 (29%) |
| Allergic sequelae of atopic dermatitis | 1/7 Asthma at 3 yr.<br>6/7 (86%) Still normal at 6 yr. | 10/20—Asthma by 4 yr.<br>4/21—Hay fever at 6 yr.<br>1/21—P.A.R. at 5 yr.<br>6/21—Normal at 6 yr. | 24/50—Asthma at 5 yr.<br>6/50—HF by 5 yr.<br>20/50—Normal at 6 yr. |
| Incidence of other allergies to cow's milk | 6/91 (7%) | 24/65 (37%) | 53/175 (30%) |
| Allergic sequelae to cow's milk allergy | None at 6 yr. | 11—Asthma at 6 yr.<br>4—HF at 5 yr.<br>1—P.A.R.<br>6—Chronic atopic dermatitis | 24/53—Asthma<br>5/53—HF<br>1/53—P.A.R. and HF |

P.A.R.—Perennial allergic rhinitis.
HF—Pollinosis (hay fever).

the incidence of atopic dermatitis and of the other allergic manifestations of allergy to cow's milk (diarrhea, colic, etc.) are similarly statistically significant. The incidence of allergic sequelae in the three groups shows an even more significant difference between the experimental and the two control groups.

## DISCUSSION

As far as the experimental group in this study has been followed, the data suggest that the complete withholding of the first food commonly ingested by a potentially allergic newborn infant (cow's milk) for a period of six to nine months may offer a hope of decreasing or minimizing the incidence of the most troublesome allergic disorders in young children. The data also lend support to the concept that an immunologic immaturity in potentially allergic children makes them more susceptible to sensitization via the gastrointestinal tract in the early months of life than toward the end of the first year.

The incidence of allergic sequelae to atopic dermatitis and other evidences of cow's milk allergy are especially interesting in view of the recent work of MacKinney and Glaser[2] which showed that 80 per cent of infants with atopic dermatitis eventually develop asthma or some other respiratory allergy. This is in marked contrast to the findings in infants fed soybean milk from birth and who nevertheless developed atopic dermatitis, of whom, as indicated in Table III, only one out of seven (14 per cent) developed a respiratory allergy. One very interesting family in this survey consisted of five children, all of whom had congenital milk allergy. In four of the children the cow's milk caused atopic dermatitis; two asthma. By contrast, the sibling, who was placed on soybean milk from birth, has not developed any major allergies by six years of age, despite the fact that he developed diarrhea from cow's milk when it was first introduced at 8 months of age. He still cannot tolerate cow's milk.

## SUMMARY AND CONCLUSIONS

Ninety-one potentially allergic infants were kept on a diet completely free from cow's milk from birth for varying periods of time up to nine months of age. Their allergic histories were compared to those of 65 siblings and 175 unrelated control children. Major allergic disease developed in 14 per cent of the experimental group; in 64 per cent of the siblings, and 52 per cent of the unrelated control group. Atopic dermatitis and other expressions of allergy to cow's milk occurred approximately four times more frequently in the two control groups. Allergic sequelae to allergy to cow's milk occurred in 16 per cent of the experimental group, 75 per cent of the siblings, and 60 per cent of the unrelated control group. These differences are all statistically significant.

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# AMINOPHYLLINE IN THE TREATMENT OF ALLERGIC DERMATOSIS. II. URTICARIA; ITS COMPARATIVE VALUE IN RELATION TO ANTIHISTAMINICS AND ACTH

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THE symptomatic treatment of allergic urticaria has always been a subject of controversy. The reason for this, it appears, is the inadequate knowledge of the pathogenesis of the urticarial wheal and its bearing on the selection of drugs for its control.

Two factors must be changed to obtain symptomatic control of urticaria: (1) the pruritus, and (2) the dermal perivascular edema.

With reference to the first it should be stated that the pruritus is due to the stimuli of the skin pain receptors and conduction by unmyelinated nerve fibers, mainly of Gasser's type "C." This sensation progresses rather slowly (one to two meters per second) leaving the skin with a tendency to remain itchy ("itchy-skin"). The cause of the latter includes the partial damage of the sensory nerve endings which lasts several days.[1] In the majority of cases of urticaria the initial pruritus is transitory, but the pruritus of the "itchy-skin" condition is more persistent and gives rise to circumstances whereby the skin reacts with pruritus to stimuli different from the one that formerly caused the sensation (mnemodermia).

The perivascular edema is also the result of the antigen-antibody reaction working upon the capillary endothelium, increasing its permeability, and allowing variable amounts of plasma protein molecules to go into the surrounding tissues, thus changing the oncotic pressure at that level and increasing even more the extravasation of fluids.

In the symptomatic control of urticaria the measures most often employed are the following: Epinephrine, calcium, autohemotherapy, proteinotherapy, antihistaminics, ACTH, and cortisone. We propose to add aminophylline to the present armamentarium.

Epinephrine modifies the changes of the capillary permeability due to antigen-antibody reaction,[2] and according to White[3] the injection of epinephrine may indirectly cause an increase of ACTH to be released which may play a part in the control of the allergic reaction. This drug acts primarily upon the edema.

Calcium, autohemotherapy and proteinotherapy are mentioned only because of their wide use. Their therapeutic value, if any, should be considered in the light of minor psychotherapy.

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Presented at the Ninth Annual Meeting of the American Academy of Allergy, Feb. 27, 1953, Boston, Mass.

Received for publication, Feb. 27, 1953.

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The introduction of antihistaminics brought hopes that the control of allergic conditions had been attained. According to Lewis' theory[4] of the triple response, the antihistaminics should answer the problem of controlling the pruritus in urticaria, if this condition were due exclusively to the liberation of histamine or "H" substances. It is true that antihistaminic drugs administered either orally or parenterally have helped us greatly, acting primarily upon the pruritic factor.

ACTH and cortisone have been supposed to act upon the mechanisms of immunity as follows:[5] 1. They may alter the relative concentrations of antigen-antibody in the tissues, thereby affecting their capacity to react. 2. They alter the tissue factors that influence the extent of combination of antigen and antibody. 3. They alter the degree of production of tissue inflammatory substances released as a result of antigen-antibody reaction. 4. They alter the capillary permeability.[6]

The use of ACTH and cortisone for the control of allergic dermatoses and particularly of urticaria has been pointed out by several investigators[7-9] and has been extremely well presented in Downing's paper.[10] These hormones act, according to the above views, upon the two factors (pruritus and edema) manifested in the urticarial wheal.

Aminophylline has been used with success in the treatment of allergic bronchial asthma.[11-14] Epstein[15] has reported the possibility of controlling pruritus in 80 per cent of a series of thirty different dermatoses with this drug. Noferi[16] reports its local anesthetizing action upon nerve endings. Segal[17] and Hambourger[18] have singled out its protecting action during anaphylactic shock caused by histamine or acetylcholine. In a former report[19] one of us (C. C.) pointed out the efficacy of this drug for the control of allergic urticaria. We have also found a paper from Salazar Mallén,[20] referring to the employment of aminophylline for the symptomatic control of various groups of allergic dermatoses. Since aminophylline belongs to the group of the methylxanthines, it has their general properties, to which we must add, being fundamentally important, their local anesthetizing, antihistaminic, and diuretic actions.[21] We have taken the following paragraph from Goodman and Gillman: "Still other mechanisms of action of aminophylline have been postulated. It has been claimed that the affinity of serum proteins for water is reduced, and evidence has also been presented to show a mobilization of fluid into the blood under the influence of this drug. These two phenomena would enhance glomerular filtration." This action suggests the possibility of reducing perivascular edema by the use of this drug.

The foregoing references indicate the double action of aminophylline, working upon both pruritus and edema, which are the pathogenic factors in the urticarial wheal.

## PROCEDURE

Twenty ambulatory patients with chronic urticaria due to food allergy were chosen, because it was possible to demonstrate in them skin sensitizing antibodies, using the passive transfer technique. The selection of the first treat-

ment to be used in each particular case was left to the order in which they came to the clinic, as follows: the first one to come would receive aminophylline; the second one, an antihistaminic drug (Thephorin) and the third one, ACTH, repeating this sequence with all the following arrivals.

In this manner, during one year (1952), it was possible for us to administer these three types of treatment alternately to each patient. None of the patients was ever informed of the substance that was given him in order to avoid suggestions as to the effects; and every one of them was submitted to a placebo treatment (water intramuscularly) as control for the results obtained.

The results were valued according to the control of the pruritus and edema. In relation to the former we depended entirely on the subjective reports of the patient to differentiate between the initial pruritus and "itchy-skin."

The latter aspect of control was followed by outlining the edge of the urticarial wheals with indelible ink and arranging that two observers, unrelated to the clinic, would record the reduction of the size of the wheals and the appearance of new wheals during the treatments. These observations were made every six hours, during the day.

Since we knew[19] that the intravenous injection of aminophylline every twelve hours, given slowly (ten minutes) was enough to control the urticarial crisis, we used a dose of 0.25 Gm. dissolved in 10 c.c. of water, every twelve hours.

Thephorin was used orally with an initial dose of 50 mg. every six hours during the first twenty-four hours, and then reduced to the minimum useful dose necessary to maintain the patient free from urticarial wheals. We chose this antihistaminic because it does not present the radical ethylenediamine in its chemical structure, which is present in aminophylline.

ACTH was used in seventeen of the twenty cases. The initial dose was 80 mg. a day, given in four doses of 20 mg. intramuscularly, every six hours, and continuing the maintenance dose needed in each case, with an average dose of 40 mg. a day. Before deciding upon the treatment, suitable tests and examinations were performed to eliminate tuberculosis, diabetes, arterial hypertension, peptic ulcer, etc. Once it had been determined that it was possible to use the hormone, Thorn's test after Randolph's technique[22] was performed to check the reduction of circulating eosinophils. ACTH was used only when the reduction of circulating eosinophils was more than 50 per cent of the initial count. In order to avoid hypopotassemia, six tablets of potassium chloride (0.135 Gm.) were given orally every day.

## RESULTS

The results obtained are given in Tables I and II. Table I shows the progress obtained in the control of pruritus. Attention is called to the fact that ACTH was used only in seventeen of the cases since the remaining three patients showed a lack of response to Thorn's test.

It can be seen from the data in Table I that aminophylline was more efficacious in controlling pruritus during the first twenty-four hours of treatment and that the majority of patients gave a positive answer within forty-

TABLE I. PRURITUS CONTROL IN URTICARIA

| DRUG | COURSES EVALUATED | NUMBER OF PATIENTS SHOWING EDEMA CONTROL IN: HOURS 12 | HOURS 12-24 | DAYS 2 | DAYS 3 | DAYS 4 | DAYS 5 | DAYS 6 | URTICARIA CONTINUED |
|---|---|---|---|---|---|---|---|---|---|
| Aminophylline | 20 | 5 | 3 | 6 | 2 | 1 | 1 | 0 | 2 |
| ACTH | 17 | 0 | 1 | 2 | 4 | 3 | 2 | 1 | 4 |
| Thephorin | 20 | 2 | 3 | 3 | 3 | 2 | 3 | 1 | 3 |
| Placebo | 20 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 16 |

TABLE II. EDEMA CONTROL IN URTICARIA

| DRUG | COURSES EVALUATED | NUMBER OF PATIENTS SHOWING EDEMA CONTROL IN: HOURS 12 | HOURS 12-24 | DAYS 2 | DAYS 3 | DAYS 4 | DAYS 5 | DAYS 6 | URTICARIA CONTINUED |
|---|---|---|---|---|---|---|---|---|---|
| Aminophylline | 20 | 5 | 3 | 6 | 2 | 1 | 1 | 0 | 2 |
| Acth | 17 | 0 | 1 | 2 | 4 | 3 | 2 | 1 | 4 |
| Thephorin | 20 | 2 | 3 | 3 | 3 | 2 | 3 | 1 | 3 |
| Placebo | 20 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 16 |

eight hours. The response to ACTH was slower. This is in accord with the reports of Cooke,[8] Feinberg,[7] and Kierland,[23] who mention the control of pruritus in their urticarial cases from the third to the fifth days of treatment with ACTH.

The antihistaminic, Thephorin, controlled pruritus in eight of the patients before forty-eight hours, which had been our experience before.[19]

The cases in which there was no improvement before six days are also recorded. We chose this period of time to judge the results since that was the average duration of urticaria in those patients. Two patients did not show any control of pruritus with aminophylline, both had had urticaria for several years. ACTH failed in four cases and Thephorin in three. The use of the placebo shows results on four of the patients but these results are fictitious if we consider that after four to five days, they might have cleared up without any treatment.

Again aminophylline proved the most effective in controlling the urticarial wheal. The edema disappeared within forty-eight hours in fifteen of the patients treated, in contrast to three patients treated with ACTH and Thephorin. In the group of controls there was not a single case in which the wheals faded spontaneously within forty-eight hours.

In every case treated with aminophylline we made sure to keep in mind the caution of injecting the drug very slowly (ten minutes or longer) avoiding in this way the fall of blood pressure that follows the rapid injection of this substance. We did not find any other manifestation of intolerance or allergy to the drug.

During the treatment with ACTH, we avoided hypopotassemia, and only one patient showed sensitization to the drug. Most of the patients showed marked euphoria and two of them presented emotional disturbances (anxiety).

Thephorin was well tolerated by the majority of the patients and only a few of them showed somnolence.

## CONCLUSIONS

1. Aminophylline compares favorably with ACTH and Thephorin in the management of allergic urticaria.

2. Aminophylline is proposed as a drug whose pharmacodynamic properties assure it a place in the symptomatic control of allergic urticaria.

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# INACTIVATION OF THE ANTIHISTAMINIC DRUGS IN THE LIVER AND IN THE GASTROINTESTINAL TRACT

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HISTAMINE is inactivated in the liver, as was shown by Ewins and Laidlaw[1] in 1910, and the finding was confirmed by Ivy and Javois,[2] Dale and Laidlaw,[3] and Koessler and Hanke.[4] Other substances are inactivated by the liver. Many of the sulfonamides are acetylated,[5, 6] sex hormones are partially destroyed[7, 8] and the liver acts on epinephrine,[9] curare,[10] insulin,[11] and on the pituitary hormones.[12]

Antihistaminic drugs are less effective when given by mouth than when given parenterally, and the question is whether the difference depends upon absorption from the gut or upon the fact that, like histamine, the antihistaminic drugs are also inactivated by the liver.

## EXPERIMENTS AND RESULTS

Experiments were made on guinea pigs. The antihistaminic potency—the ability to neutralize histamine—was measured by determining the protective activity of the drug against doses of histamine which were lethal for untreated animals. Gastrointestinal absorption was tested by comparing the effect of oral administration with the effect of intraperitoneal injection of the antihistaminic; liver inactivation was tested by comparing the effect of intraportal as against intrajugular injections.

For our experiments we used the following antihistaminics: Neo-Antergan, Multergan, Phenergan, Neohetramine, Trimeton, Hista-3, "Life" (a mixture of 40 per cent of Neo-Antergan, 30 per cent of Trimeton, and 30 per cent of Neohetramine), Thephorin, and Antistine.

In the experiments, a constant dose of the antihistaminic drug was given at the start by jugular vein in the first series and by a tributary of the portal vein in the second. Five minutes later histamine was injected, and then injected again at intervals of three minutes apart, increasing the quantities until the final dose killed the animal. In one group, the test dose of histamine was injected into the jugular vein; in the other it was injected into a tributary of the portal vein. The protective potency of the antihistaminic is expressed as multiples of the dose of histamine which is fatal for normal, unprotected animals.

Table I shows that when histamine is injected into the jugular vein of the guinea pig, the $DL_{50}$ dose is 0.60 mg./kg., but when it is injected into the portal vein, the $DL_{50}$ dose is as much as 2.7 mg./kg. of guinea pig. This result shows that the histamine has been inactivated by the liver.

Table II shows that when the guinea pigs are treated before the histamine by injecting antihistaminic drugs into the jugular vein in one fixed dose, the

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Received for publication Feb. 26, 1953.

TABLE I. LETHAL DOSES OF HISTAMINE (GROUP 1)

| ROUTE OF ADMINISTRATION | NO. GUINEA PIGS | $DL_{50}$ MG./KG. | S.E.* |
|---|---|---|---|
| Intrajugular injection (Subgroup I) | 32 | 0.6 | 0.07 |
| Intraportal injection (Subgroup II) | 35 | 2.7 | 0.42 |

*Standard error.

mean lethal dose for histamine (mLD) is much larger than in the unprotected controls: indeed, Neo-Antergan will protect against over one hundred fatal doses of histamine while for other drugs the range of protection falls off to 50 or even to only 8 times the fatal dose.

TABLE II. MEAN LETHAL DOSE OF HISTAMINE AFTER ADMINISTRATION OF THE ANTIHISTAMINIC INTO THE JUGULAR VEIN (GROUP 2)

| ANTIHISTAMINIC INTRAJUGULAR (0.030 MG./KG.) | HISTAMINE | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | INTRAJUGULAR (SUBGROUP III) | | | | INTRAPORTAL (SUBGROUP IV) | | | |
| | NO. GUINEA PIGS | mLD* MG./KG. | S.E.† | P mLD/ 0.6‡ | NO. GUINEA PIGS | mLD* MG./KG. | S.E.† | P mLD/ 2.7‡ |
| Neo-Antergan | 22 | 65.0 | 5.2 | 108.0 | 20 | 288.0 | 50.7 | 101.0 |
| Multergan | 8 | 58.0 | 6.8 | 97.0 | – | – | – | – |
| Hista-3§ | 20 | 31.0 | 4.2 | 51.6 | 15 | 148.0 | 19.6 | 55.0 |
| Phenergan | 10 | 27.6 | 3.1 | 46.0 | 6 | 113.0 | 16.2 | 42.0 |
| Neohetramine | 20 | 9.9 | 0.5 | 16.5 | 8 | 39.4 | 3.7 | 14.0 |
| Trimeton | 12 | 7.1 | 0.3 | 11.8 | 10 | 34.0 | 8.2 | 12.6 |
| Thephorin | 10 | 4.9 | 0.6 | 8.2 | 10 | 22.0 | 4.0 | 8.2 |

*Mean lethal dose (average from all animals of group).
†Standard error.
‡Antihistaminic potency.
§Hista-3 is a mixture of 40 per cent Neo-Antergan, 30 per cent Trimeton, and 30 per cent Neohetramine.

Table III shows the results when the antihistaminic treatment is given by portal vein. This time the protective potency is less than 10 times, and the difference between intrajugular and intraportal treatment is striking. In neither of these two experiments does it make any large difference whether the histamine challenge is given by the jugular or the portal system, and, according to the results in normal animals, as shown in Table I, that fact is disconcerting.

TABLE III. MEAN LETHAL DOSE OF HISTAMINE AFTER ADMINISTRATION OF THE ANTIHISTAMINIC INTO THE PORTAL VEIN (GROUP 3)

| ANTIHISTAMINIC INTRAPORTAL (0.030 MG./KG.) | HISTAMINE | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | INTRAJUGULAR (SUBGROUP V) | | | | INTRAPORTAL (SUBGROUP VI) | | | |
| | NO. GUINEA PIGS | mLD* MG./KG. | S.E.† | P mLD/ 0.6‡ | NO. GUINEA PIGS | mLD* MG./KG. | S.E.† | P mLD/ 2.7‡ |
| Neo-Antergan | 15 | 6.2 | 2.3 | 10.3 | 10 | 18.0 | 3.6 | 6.7 |
| Multergan | 6 | 5.8 | 0.8 | 9.7 | – | – | – | – |
| Phenergan | 10 | 3.6 | 0.9 | 6.0 | 8 | 16.8 | 2.3 | 6.2 |
| Hista-3 | 15 | 5.5 | 0.5 | 9.2 | 10 | 25.0 | 2.2 | 9.3 |
| Neohetramine | 10 | 2.2 | 0.08 | 3.7 | 10 | 9.5 | 0.6 | 3.5 |
| Trimeton | 12 | 1.9 | 0.02 | 3.2 | 9 | 6.7 | 0.5 | 2.5 |
| Thephorin | 12 | 3.1 | 0.1 | 5.0 | 10 | 16.0 | 1.1 | 5.9 |

*Mean lethal dose (average from all animals of group).
†Standard error.
‡Antihistaminic potency.

TABLE IV. GAIN OR LOSS OF ANTIHISTAMINIC POTENCY BY THE DRUGS' PASSAGE THROUGH THE LIVER

| NAME OF THE ANTIHISTAMINICS | ANTIHISTAMINIC BY JUGULAR VEIN HISTAMINE JUGULAR III | ANTIHISTAMINIC BY PORTAL SYST. HISTAMINE PORTAL IV | ANTIHISTAMINIC BY JUGULAR VEIN HISTAMINE JUGULAR V | ANTIHISTAMINIC BY PORTAL SYST. HISTAMINE PORTAL VI | PERCENTAGE OF PROTECTIVE POTENCY LOSS BY ANTIHISTAMINIC LIVER PASSAGE III/V 1 | III/VI 2 | IV/VI 3 | GAIN BY HISTAMINE LIVER PASSAGE III/IV 4 | V/VI 5 |
|---|---|---|---|---|---|---|---|---|---|
| Neo-Antergan | 108.0 | 101.0 | 10.3 | 9.3 | 95.5 | 91.0 | 90.7 | -6.3 | -9.0 |
| Multergan | 97.0 | - | 9.7 | - | 90.0 | - | - | - | - |
| Hista-3 | 51.6 | 55.0 | 9.2 | 9.3 | 82.2 | 82.0 | 87.0 | +6.8 | +1.0 |
| Phenergan | 46.0 | 42.0 | 6.0 | 6.2 | 87.0 | 87.5 | 85.3 | -8.5 | +3.0 |
| Neohetramine | 16.5 | 14.0 | 3.7 | 3.5 | 77.8 | 80.7 | 77.2 | -15.0 | -5.5 |
| Trimeton | 11.8 | 12.6 | 3.2 | 2.5 | 73.0 | 80.1 | 80.2 | +7.0 | -12.7 |
| Thephorin | 8.2 | 8.2 | 5.0 | 5.9 | 39.0 | 23.0 | 28.0 | 0 | +15.0 |

When, however, the percentage of protection is compared (Table IV), it is found that the jugular route is more effective, and one can conclude that just as histamine itself is inactivated by the liver, so also are the antihistaminic drugs inactivated by the liver. Furthermore, when the time interval be-

TABLE V. MEAN LETHAL DOSE OF HISTAMINE (BY JUGULAR VEIN) AFTER DIFFERENT INTERVALS OF ADMINISTRATION OF THE ANTIHISTAMINIC (BY PORTAL SYSTEM)

| MINUTES OF INTERVAL | NEO-ANTERGAN NO. GUINEA PIGS | mLD* MG./KG. | mLD* S.E.† | P mLD/ 0.6‡ | THEPHORIN NO. GUINEA PIGS | mLD* MG./KG. | mLD* S.E.† | P mLD/ 0.6 ‡ |
|---|---|---|---|---|---|---|---|---|
| 5 | 14 | 6.3 | 2.1 | 10.3 | 12 | 3.1 | 0.1 | 5.0 |
| 10 | 4 | 4.2 | 1.9 | 7.0 | 4 | 2.0 | 0.1 | 3.3 |
| 15 | 4 | 3.6 | 0.5 | 6.0 | 3 | 1.4 | 0.09 | 2.3 |
| 20 | 4 | 3.0 | 0 | 5.0 | 3 | 1.4 | 0.09 | 2.3 |

*Mean lethal dose (average from all animals of group).
†Standard error.
‡Antihistaminic potency.

tween antihistamine dose and histamine challenge is lengthened, the protection becomes less, because of the action of the liver (Table V). With more time, more antihistamine is destroyed: it takes less histamine to kill the animal.

TABLE VI. COMPARATIVE PROTECTION OF GUINEA PIGS AGAINST CONVULSIONS WHEN EXPOSED TO HISTAMINE SPRAY AFTER ADMINISTRATION OF THE SAME DOSE OF THE ANTIHISTAMINIC BY MOUTH AND INTRAPERITONEALLY

| ANTIHISTAMINIC | BY MOUTH DOSE MG./KG. | PROTECTED /USED* | $MH_t$† AVERAGE | S.E.‡ | INTRAPERITONEALLY DOSE MG./KG. | PROTECTED /USED* | $MH_t$† AVERAGE | S.E.‡ | P§ |
|---|---|---|---|---|---|---|---|---|---|
| Neo-Antergan | 0.250 | 16/23 | 7'10" | 1'06" | 0.250 | 13/19 | 7'20" | 1'20" | 0.9 |
| Multergan | 0.250 | 11/15 | 7'00" | 1'15" | 0.250 | 9/15 | 5'50" | 1'05" | 0.5 |
| Hista-3 | 0.250 | 16/22 | 7'40" | 1'30" | 0.250 | 15/21 | 8'10" | 1'45" | 0.8 |
| Phenergan | 0.300 | 12/20 | 6'50" | 0'40" | 0.300 | 11/20 | 5'55" | 0'50" | 0.6 |
| Neohetramine | 3.500 | 8/15 | 7'05" | 1'20" | 3.500 | 7/15 | 6'10" | 1'35" | 0.8 |
| Trimeton | 3.000 | 12/17 | 6'45" | 1'05" | 3.000 | 13/18 | 7'00" | 1'25" | 0.8 |
| Thephorin | 2.000 | 8/15 | 7'15" | 1'10" | 2.000 | 8/15 | 7'40" | 1'50" | 0.9 |
| Histadyl | 4.000 | 6/10 | 7'00" | 1'15" | 4.000 | 5/10 | 6'22" | 1'20" | 0.8 |
| Antistine | 4.000 | 3/10 | 6'00" | 1'30" | 4.000 | 3/11 | 5'25" | 0'58" | 0.7 |

*Animals protected for ten minutes/total of animals treated.
†Modified histamine time, in minutes.
‡Standard error.
§Probability value of the statistical significance of the difference between the two $MH_t$.

In a second series of experiments, histamine was administered by aerosol in a closed "asthma chamber." The onset of hypoxemic convulsions was used as the end point, and the time interval between the start of the inhalation and the onset of symptoms, the "modified histamine time," was a measure of activity (Table VI).

When the animals were prepared by giving antihistaminic drugs in solution by mouth, they could tolerate the histamine spray for an average of about seven minutes as compared with one minute and forty-five seconds for the unprepared controls. When the drug was given by intraperitoneal injection, the times were about the same. This shows that the drugs are not inactivated in the gut. One should note that for protection the doses by mouth and by the intraperitoneal route must be 3 to 5 times larger than when they are given subcutaneously, as we have shown in a previous article,[13] and that is another indication of the inactivation by the liver.

## CONCLUSIONS

1. In guinea pigs, about 70 per cent of the histamine which reaches the liver by way of the portal vein is inactivated by that organ.

2. All seven antihistaminics studied (Neo-Antergan, Multergan, Hista-3, Phenergan, Neohetramine, Trimeton, and Thephorin) are inactivated in a large proportion by the liver. The percentage of hepatic inactivation varies with substance and dose, but, in general, amounts to from 70 per cent to 90 per cent of the drug. Thephorin was least inactivated (39 per cent) while Neo-Antergan and Multergan suffered the highest hepatic inactivation (90 per cent).

3. The antihistaminics are not inactivated in the gastrointestinal tract.

4. When given intraperitoneally, the drugs are absorbed by the portal system.

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# SENSITIZATION OF NASAL MUCOUS MEMBRANES TO BACTERIA

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BACTERIAL infection may cause "atopic" symptoms in children. Chobot[1] and his associates found it was an important cause of asthma in 87 per cent of the children they studied. In 50 per cent it was a contributory factor along with clinical sensitizations to foods and inhalants, but in 30 per cent was the only cause they could discover. Not all atopic children have asthma with their infections.[2] Some have only rhinitis and others rhinitis and bronchitis without frank asthmatic attacks. The symptoms of infection have been observed in children after colds which they have had in the winter. In some cases long intervals free of symptoms have intervened between epidemic infections, but in most cases interepidemic bacterial infections have been frequent all winter long. These infections have caused symptoms in either one of two ways: in some patients the respiratory mucous membranes have apparently been sensitized to bacteria, and in others infection has merely been an irritant which has enhanced the symptoms of a latent sensitization to some of the common allergens.[3]

The common respiratory bacteria, *Staphylococcus aureus,* hemolytic streptococcus, pneumococcus, *Streptococcus viridans, Micrococcus catarrhalis,* and the influenza bacillus have been cultured from the nasopharynx of patients with colds and with asthmatic attacks. The floras of these patients have always been fluid, never static.[4] When cultures have been made from a patient in successive attacks of asthma occurring during the winter, the gram-positive cocci have been found in increasing numbers until late spring, then less and less frequently until August. This cycle has been repeated year after year. In cultures made during successive attacks in one of these patients, the same species or strains have not always been found in the nasopharynx in different attacks. In bacterial epidemic infections of the upper respiratory tract other than infections associated with asthma or colds, about one-half of the complications have been caused by some strain which has supplanted the one causing the initial infection.[5] Nor have the floras of these complications always been stable. The species of bacteria found in sinus infections change, sometimes just as the sinus infection has become subacute, and often even in the established chronic infections.[6, 7] Apparently bacteria cultured from the nasopharynx or foci of infection during one allergic attack may not be recovered in similar attacks occurring later. It has been impossible to predict just what the nasopharyngeal floras in these subsequent attacks may be.

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Received for publication, April 8, 1953.

Because the floras have been so variable in patients with atopic respiratory disease, strains of all species of bacteria, enumerated in the preceding paragraph, commonly recovered from patients during infections causing allergic attacks have been included in therapeutic preparations.[4] At times substitutions or additions have been made from autogenous cultures. Originally vaccines were used, but a mixture of filtrates of old cultures of the several species mentioned has been employed recently since they were found therapeutically superior to vaccines in comparative studies.

Although respiratory reactions have followed inoculations with vaccines, they have occurred more frequently at some level of dosage during therapeutic injections of filtrate. Depending on the degree of tolerance inoculations have been started in the early autumn with either a 1:1,000, a 1:100, or a 1:10 dilution of the mixed culture filtrates. The doses have then been increased gradually, but as rapidly as possible without causing severe local or focal reactions, up to one milliliter of the undiluted mixture. Most children have tolerated these inoculations very well, and well-defined reactions have not occurred until mid- or late winter when the doses were large. During this season when infections have been prevalent, it has been difficult to distinguish a reaction to an inoculation from a mild infection.

Although most children have been reasonably tolerant to fairly large doses of filtrate (0.5 ml. of undiluted mixture), about one in fifteen has had mild upper respiratory reactions to smaller doses. A few have been very sensitive with a critical dosage level which has been difficult to break through. As little as 0.05 ml. of a 1:100 dilution has caused upper respiratory reactions with mild fever. Reactions in the very sensitive children have occurred early in the fall when inoculations were first begun. At this time when respiratory infections have been less frequent than in the winter months, in children with a "critical level of dosage" several reactions have occurred before this level was broken through so that the doses could be increased. In a number of patients the inoculations have been given irregularly and control material has also been injected, so no question could be entertained regarding the causal relationship of the inoculations to the reactions.

In the last few years, observations to demonstrate the sensitization of the nasal mucous membranes to filtrates were started on thirty children. Only nine of the series were completed. These nine patients were seen in the spring and, because they had no symptoms during the summer, were advised to wait until September for treatment. When they were first seen all had had rhinitis during the past winter with exacerbations of infection at two- or three-week intervals. None had apparent clinical sensitizations to the common allergens. The mucous membranes were pale, swollen and boggy, and covered with a seromucoid discharge containing eosinophils. Six had had repeated attacks of bronchitis, and in three asthmatic râles had been heard during bronchitic attacks. Three of the children had had their tonsils and adenoids removed the previous spring. Cultures were made and filtrates were prepared in September. If reactions occurred following the first two or

three inoculations, a control injection was given followed by an additional dose of filtrate. Reactions were first noticed six to eight hours after an inoculation. The nasal passages were occluded and the membranes swollen and covered with a seromucoid discharge. There might be a cough, mild angina, and occasionally headache, and slight fever. The symptoms subsided in twenty-four to forty-eight hours. The significant reactions in the children have been tabulated and are shown in Table I.

TABLE I. UPPER RESPIRATORY REACTIONS TO FILTRATES

| PATIENT AGE | REACTIONS FIRST DOSE DILUTION REACTION | SECOND DAYS | SECOND DOSE DILUTION REACTION | THIRD DAYS | THIRD DOSE DILUTION REACTION | FOURTH DAYS | FOURTH DOSE DILUTION REACTION | *SUBSTITUTIONS AND ADDITIONS TO STOCK MIXTURE OF FILTRATES |
|---|---|---|---|---|---|---|---|---|
| J. S.<br>3 yr. | 0.3 ml.<br>1 to 100<br>++ | 8 | 0.4 ml.<br>1 to 100<br>+++ | 9 | *Culture*†<br>*Medium, 0.1*<br>– | 5 | 0.5 ml.<br>1 to 100<br>++ | 1,*6* |
| M. R.<br>4 yr. | 0.7 ml.<br>1 to 100<br>+++ | 6 | *Culture*†<br>*Medium, 0.1*<br>– | 7 | 0.7 ml.<br>1 to 100<br>++ | | | 3 |
| G. M.<br>5 yr. | 0.1 ml.<br>1 to 10<br>++ | 9 | 0.5 ml.<br>1 to 100<br>+ | 5 | *Tetanus*†<br>*Toxoid, 0.1*<br>– | 8 | 0.1 ml.<br>1 to 10<br>++ | 3 |
| F. A. R.<br>5 yr. | 0.8 ml.<br>1 to 100<br>+++ | 7 | 0.5 ml.<br>1 to 100<br>+ | 8 | *Tetanus*†<br>*Toxoid, 0.1*<br>– | 7 | 0.8 ml.<br>1 to 100<br>++ | 4,*6* |
| T. A. R.<br>5 yr. | 0.2 ml.<br>1 to 100<br>++ | 7 | *Tetanus*†<br>*Toxoid, 0.1*<br>– | 8 | 0.3 ml.<br>1 to 100<br>++ | | | 4,*6* |
| M. L.<br>6 yr. | 0.3 ml.<br>1 to 100<br>+++ | 10 | 0.2 ml.<br>1 to 100<br>++ | 11 | 0.3 ml.<br>1 to 100<br>+ | 5 | *Pertussis*,<br>*0.1*<br>– | |
| F. U.<br>3 yr. | 0.06 ml.<br>1 to 10<br>++ | 7 | 0.4 ml.<br>1 to 100<br>+ | 7 | *Diph.*†<br>*Toxoid, 0.1*<br>– | | 0.1 ml.<br>1 to 10<br>+++ | |
| J. S.<br>3 yr. | 0.8 ml.<br>1 to 100<br>+ | 6 | *Tetanus*†<br>*Toxoid, 0.1*<br>– | 14 | 0.1 ml.<br>1 to 10<br>+ | 14 | 0.3 ml.<br>1 to 10<br>++ | *6* |
| T. L.<br>5 yr. | 0.3 ml.<br>1 to 10<br>+++ | 9 | 0.3 ml.<br>1 to 10<br>+ | 6 | 0.3 ml.<br>1 to 10<br>++ | 10 | *Diph.*†<br>*Toxoid, 0.2*<br>– | 3 |

*A mixture of stock filtrates from cultures of selected strains of (1) *Streptococcus hemolyticus*, (2) *Streptococcus viridans*, (3) *Staphylococcus aureus*, (4) pneumococcus II, and (5) *Neisseria catarrhalis* was used in these inoculations. Substitutions of autogenous filtrates are indicated by the number. *Hemophilus influenzae* was added only if found in cultures. This addition, *6*, is italicized.

†Control inoculations are also in italics.

## DISCUSSION

The upper respiratory mucous membranes of these children are hypersensitive to bacterial products in the polyvalent filtrates. The immunologic processes, such as immunity and sensitization, are apparently similar in these membranes to those in the skin. Two of the bacteria recovered frequently in the nasopharyngeal cultures cause recurrent dermal infections with sensitization of the affected areas to the nucleoproteins and filtrates of cultures

of these bacteria.[8] They are *Streptococcus hemolyticus* causing erysipelas and *Staphylococcus aureus* found in certain erysipeloid infections. Erysipelas, which is more common and consequently has been studied more thoroughly, recurs in about 20 per cent of patients. There may be one, two, three, or even more reinfections of the same site. Local immunity to *Streptococcus hemolyticus* is effective for about three months to the type of the strain causing the infection.[9] In these patients if the focus still harbors these cocci, reinfections may occur when this immunity is no longer protective. Infection advances along a raised margin with an accompanying severe general reaction, but successive attacks provoke milder and milder constitutional symptoms even though the local inflammation seems just as severe.

Bacteria are numerous in sections and cultures of the skin in the first two or three infections, but subsequently the inflammatory reaction is more diffuse. Then the streptococci are sparse and seldom can be found or recovered. The affected tissues are apparently so hypersensitive, that the sparse growth of a few avirulent bacteria may cause a disproportionately severe reaction. Each attack apparently leaves the area highly sensitized, because within a few days after the inflammation has resolved local cutaneous reactions have been positive and distant inoculations with streptococcal nucleoproteins or filtrates have caused regional edema, redness, and swelling. Similar reactions have been gotten with nucleoproteins and filtrates of staphylococcus in staphylococcal erysipeloid infections. Although chronic edema has been observed following several recurrences in skin which was normal when first infected, areas which are already edematous are especially susceptible to infection. Spontaneous reinfections may occur in these edematous tissues after the focus is sterile, suggesting that bacteria may lie inactive in the tissues between attacks. In patients specifically hyposensitized with filtrates of streptococcus or staphylococcus, reinfections have first been less frequent and severe and finally have ceased. Similar inoculations with polyvalent filtrates have prevented interepidemic rhinitis and bronchitis in children.[2]

In patients who are not atopic, filtrates of the cultures of the bacteria included in the polyvalent mixture, as well as the bacterial nucleoproteins, cause delayed tuberculin-like reactions if injected intradermally in those who are sensitive. These reactions vary from year to year, season to season, and month to month. In the late winter and spring more species cause reactions and the reactions are more intense than in the summer and early fall. The frequency with which positive reactions are obtained and their intensity has paralleled the incidence of the different species in nasopharyngeal cultures, but in these cultures the species vary greatly, and often no one species is found in such predominance that its occurrence in a culture can be correlated with the corresponding dermal reaction. Only when a species has been predominant and has been the cause of subacute or acute symptoms can a definite correlation be established. Minimal and subclinical infections must occur during the course of a winter. The behavior of these dermal reactions—the haphazard appearance and disappearance of positive reactions to different

bacteria, the occurrence of positive tests remaining positive for only a few weeks or two or three months, the varying intensity of those lasting through a winter, and the seasonal variations of those which recur year after year—can be explained in no other way.[10]

An immediate wheal and flare has been observed occasionally in atopic patients with staphylococcus[11] tetanus, diphtheria toxins or toxoids and with tuberculin.[12] The antibody which passively transfers the immediate reaction to diphtheria toxin appears to be similar to the "passive transfer antibody" in the serum of patients with pollenosis.[13] Although typical immediate reactions have been observed with the nucleoproteins and atoxic filtrates of the upper respiratory bacteria in atopic patients, edema at the site of a dermal injection intermediate in type between the immediate wheal and flare reaction and the delayed tuberculin-like reaction has been seen more frequently. This intermediate reaction has appeared to be a modified tuberculin reaction and has not been passively transferred with the patient's serum.[10] All three reactions, the wheal, the intermediate, and the delayed, occur in atopic patients, but the last two are by far the most common.

Local hypersensitization of the mucous membranes and cutaneous sensitizations to the nucleoproteins and culture filtrates of the common respiratory bacteria seem analogous in many ways to sensitizations occurring with tubercle bacilli. Infections with tubercle bacilli, even though minimal and subclinical, cause permanent sensitizations. This sensitization is most intense at the site of the lesion, but other sites, including the skin, become sensitive to a lesser degree. The sensitization varies with the activity of the infection, and can be detected with purified proteins obtained from the bacillary bodies or with tuberculin prepared from heated old cultures or from unheated culture, filtrates. Although inoculations of living or dead bacilli or of proteins carefully extracted from the bacilli will sensitize, especially when injected with adjuvants, the tuberculins, both O.T. and N.T., are nonantigenic. However, these tuberculins cause local and focal reactions in sensitive patients and will desensitize them if given in gradually increasing doses. These tuberculins are prepared from old cultures and some are heated. It is probable that they are not antigenic because the bacterial proteins are denatured or largely disintegrated.

The proteins isolated from tubercle bacilli contain more or less nucleic acid[14] and are presumably nucleoproteins or degraded and disintegrated nucleoprotein fractions. These protein fractions as well as those isolated from unheated culture filtrates if given in sufficient concentration[15] are antigenic and will sensitize and give typical tuberculin cutaneous reactions.[16] Presumably these reactions are similar to those produced with pneumococcus nucleoprotein,[17] and with filtrates of hemolytic streptococcus[18] and *Staphylococcus aureus* which will sensitize, and then if inoculations are continued cause desensitization. These reactions seem to be characteristic of the bacterial nucleoproteins or of disintegrated, degraded, or denatured protein fractions in the filtrates.

## CONCLUSIONS

In previous studies of recurrent infections of the skin with *Streptococcus hemolyticus* and *Staphylococcus aureus* the infected areas have been hypersensitive to the nucleoproteins and culture filtrates of these bacteria. Evidence is offered suggesting that the nasal mucous membranes of atopic children with recurrent bacterial rhinitis are similarly sensitized to the upper respiratory bacteria.

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# CUTANEOUS SENSITIZATION TO BLUE-GREEN ALGAE

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ALLERGIC dermatitis produced on contact to vegetation in susceptible individuals is a common occurrence and a large variety of plants, pollens, flowers, plant oils, leaves, and woods have been incriminated as proved etiologic agents. However, we have been unable to find any reference in the literature to cutaneous sensitization to aquatic vegetation, although there is no reason to suspect that organic compounds of this source are exempt from the capacity to sensitize the human skin under suitable conditions of exposure and contact. Therefore, our recent experience with a patient who manifested an allergic dermatitis apparently due to contact type sensitivity to a blue-green alga was felt to be worthy of reporting. To our knowledge there have been no previous case reports of epidermal sensitization to a member of the Myxophyceae.

## CASE REPORT

A 6-year-old white girl was first seen on June 25, 1952, for evaluation of a dermatitis. The history, given by her mother, was that the present skin eruption simply represented another exacerbation of a recurrent pruritic seasonal dermatitis of 3 years' duration. The eruption, since the initial onset at 3 years of age, had always been limited in its occurrence to the months of June, July, and August. Its appearance always followed swimming in a lake in Pennsylvania where she spent the summer, and on one occasion it occurred after bathing in a Canadian lake during her only visit to that region. It had never been observed after swimming in an artificial pool or ocean water, or following a bath or a shower, and never appeared in other bathers swimming in the same water either at the same or at other times of the day. Apparently it was unrelated to temperature, exposure to sunlight, or playing at the water's edge in a bathing suit without actual body contact with the lake water. The onset of the dermatitis usually occurred within one to two hours following swimming and was limited to the face, neck, shoulders, upper chest, and extremities and had never been observed on areas covered by the bathing suit. The referring physician, who had previously observed the typical course of the dermatitis, had described its appearance as erythematous-papulo-vesicular which on occasions had progressed to oozing and crusting. Provided there was no further contact with the lake water the eruption would usually subside spontaneously within two weeks and the skin would remain clear until the next episode of lake bathing. The present episode of dermatitis, the second to occur that summer, began rather typically two days previously and was first observed about two hours after bathing in the lake.

Physical examination revealed a well-developed, well-nourished 6-year-old white girl, not appearing acutely or chronically ill. There was a skin eruption limited to the thighs, lower legs, arms, upper anterior chest, neck, ears, and abdomen. The dermatitis was characterized by large irregular patches of erythema of the involved skin with pinhead-sized red papules and slightly larger vesicles. The remainder of the findings on physical examination were all within normal limits.

A survey was made of the lake (Lake Carey, Pa.) and specimens were taken of the lake water, the aquatic vegetation, and vegetation from the shore and along the path to the lake front. Appropriate patch tests of these were applied according to standard procedure to normal and previously uninvolved skin of the back. The following results were noted after removing the patches forty-eight hours after their application: (1) all aquatic

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Received for publication March 16, 1953.

vegetation (except algae) indigenous to the lake in question, negative; (2) filtered lake water, negative; (3) tap water, negative; (4) lake water sediment, 4+. This patch test resulted in an area of erythematous-papulo-vesicular dermatitis which was an exact duplication of the originally observed dermatitis. The application of ice to the skin for fifteen minutes did not result in any immediate or delayed skin reaction.

A biologic microscopic survey of the sample of lake water revealed the plankton forms found to be few, but one form, a species of Anabena, was present in tremendous numbers. The general color of the lake was green from the population of Anabena. Associated with it in the sample were small numbers of other blue-greens in the genera Coelosphaerium, Microcystis, and Aphanocapsa. A few individuals of a desmid, Staurastrum, and a ubiquitous dinoflagellate, *Ceratium hirundenella*, were present. Of the animal forms present none was in great numbers. The most common was a rotifer, *Nothalca longispina*. *Keratella cochlearis* and *Polyarthra trigla* were also present. Of the plankton crustaceans there were a few in the genera Bosmina, Daphnia, and Cyclops.

Results of further patch testing with materials applied for forty-eight hours were as follows: (1) Anabena filtered from lake water, 4+; (2) chloroform solution of Anabena, 3+; (3) phycocyanin pigment (extracted in crystalline form by ammonium sulfate from a solution of Anabena in chloroform water[1]), 3+; (4) filtrate from (3), negative; (5) hydroxylamine (liberated by decomposing blue-green algae[3, 4]) 0.1 per cent aqueous solution, 4+.

Twenty-five normal subjects served as controls in evaluating the results of patch tests. All were tested to identical samples and quantities of the materials that produced positive tests in the patient. All subjects showed negative reactions to Anabena, a chloroform water solution of Anabena, and phycocyanin. More than one-half of control subjects showed 1+ to 2+ reactions to hydroxylamine 0.1 per cent aqueous solution.

It was later verified that the dermatitis could be produced at will in the child by swimming in the lake water that contained a "bloom" of the blue-green algae whereas other individuals were unaffected under identical conditions.

## DISCUSSION

The greenish discoloration of lakes, ponds, and stagnant bodies of water is a familiar sight during the warm summer months even though the cause may not be generally recognized as a "water bloom" due to accumulations of tremendous populations of blue-green algae near the surface. The results of the decay of these algae have been recognized as being of economic importance. Disagreeable tastes and odors are imparted to the drinking water.[2] Increased bacterial flora result from the decay and this together with the living plants depletes the oxygen requirement for fish and other aquatic animals.[3] Poisonous substances such as hydroxylamine produced from the decay of proteins with which blue-green algae are supplied may kill fish[3] and may result in the poisoning of cattle and other domestic animals that drink this water.[3, 4]

Aquatic vegetation may under certain conditions be of etiologic importance in allergic reactions. Heise has reported on the allergenicity of certain blue-green algae, Oscillatoriaceae[5] and Microcystis,[6] in the production of hay fever-like symptoms in swimmers. Though some members of the group Phycomycetes are the only predominantly aquatic fungi, other fungi of proved allergenicity, such as Penicillium, can be found in flocculent tufts in tanks and swimming pools and Fusarium capable of development when submerged is thus occasionally encountered in nature.[7]

Though this is believed to be the first case reported of contact type dermatitis due to cutaneous sensitization to a blue-green alga, Anabena, there had

never been any reason to suspect that organic chemical compounds found in aquatic vegetation possessed any special characteristics to exempt them from this potential capacity. A possible reason for this infrequency is probably related to conditions concerned with exposure and contact. The opportunity for body contact with the offending form of aquatic vegetation would not necessarily be the only prerequisite, for contact would have to be of sufficient duration to permit cutaneous absorption and compensate for the dilution factor and the skin washing effect of the water on the allergen. In this regard it is noteworthy that of the sites of involvement of the dermatitis where there had been contact with the Anabena, the most marked lesions were found on the upper thighs and behind the ears where it had been observed that the algae had been caught under the edge of the bathing suit and by the hair and held there in prolonged contact with the skin.

The events related in the history, the appearance and distribution of the lesion, the ability to reproduce the dermatitis on exposure to the suspected etiologic agent, and the proof offered by positive patch tests in the patient, with negative reactions obtained on testing 25 normal controls, implicate Anabena as the etiologic agent in this instance of contact dermatitis. Anabena,[2] a blue-green alga, is botanically classified in Phylum, Myxophyceae; Suborder, Heterocystineae; Family, Nostocaceae; Genus, Anabena, and is found in the form of short, curved or straight chains of cells. Microcystis and Oscillatoriaceae, algae described by Heise[5, 6] as etiologic allergens in the production of hay fever-like symptoms, are also Myxophyceae but belong to different families from Anabena. Our plankton samples were practically pure cultures of Anabena containing very few of the algae referred to by Heise,[5, 6] who makes no mention of finding Anabena in his samples.

On further analysis it was found that at least one of the constituents of this alga responsible for this contact dermatitis was phycocyanin. This organic compound of the chemical formula $C_{48}H_{120}O_{18}N_{12}$ is the predominant blue pigment of the blue-green alga[2] which distinguishes it from other forms of plant life that contain predominantly chlorophyll. The fact that on application to the skin, phycocyanin reproduced the lesion in the patient whereas control patch tests with phycocyanin on normal individuals were negative suggests that its mechanism of action was a contact allergen rather than a primary irritant.

Since hydroxylamine ($NH_2OH$) is liberated from the decaying proteins of large "water blooms" of blue-green algae in amounts sufficient to be injurious to fish and domestic animals,[3, 4] the possibility was considered that this too might be important in the production of dermatitis. Accordingly, a patch test of a solution of this material was applied to the skin of the patient and resulted in a positive reaction. However, similar patch tests with a 0.1 per cent aqueous solution of this base resulted in positive reactions on more than one-half of tested normal individuals, thereby indicating its primary irritant action on the skin. Thus, if this chemical compound found in solution in the lake water was a causative factor in the production of dermatitis any mechanism of action as an allergen is not apparent. In this regard it is noteworthy that we are aware of conjunctivitis occurring in swimmers in lakes exhibiting a "water

bloom'' of blue-green algae. Among etiologic possibilities this could arise as an allergic conjunctivitis in atopic individuals sensitive to the forms of algae as reported by Heise[5, 6] or sensitivity to other aquatic fungi of proved allergenicity such as Penicillium and Fusarium, or perhaps to a contact type of ocular sensitivity to Anabena similar to the contact skin sensitivity of the patient reported here. However, the possibility of a primary irritant action on the conjunctiva of a significant concentration of hydroxylamine in solution in the lake water must be considered and evaluated.

In evaluating the etiology of the dermatitis of the patient reported here several other possibilities were of necessity considered in the differential diagnosis. These included (1) the aggravating effects of water on a pre- or coexisting dermatitis; (2) a seasonal atopic type dermatitis with climatic etiologic factors; (3) a seasonal skin sensitivity to ultraviolet rays; (4) contact dermatitis due to sensitivity to the materials or dye in the bathing suit; (5) contact dermatitis due to plants found along the lake shore, e.g., poison ivy, pollens, etc.; (6) a physical (cold-temperature) allergy; (7) chigger or other insect bites; (8) ''swimmers' itch'' due to schistosome cercariae. All of the above possibilities were ruled out. There was no history of any pre-existing dermatitis. Skin lesions were not observed if the patient only wore a bathing suit, exposed herself to sunlight, played along the shore among various types of grasses and weeds, and did not go in swimming in the lake. Furthermore, the distribution of the dermatitis was limited to exposed areas of the body not covered by the bathing suit and skin lesions did not appear following swimming in other types of water, bathing in fresh running cold or warm tap water, and skin contact with ice or exposure of the skin to sudden changes of water of different temperature gradients.

''Swimmers' itch,'' a maculopapular-urticarial eruption, as a reaction to the skin penetration of schistosome cercaria, is a well-known entity in the lakes of Michigan, Minnesota, and Wisconsin where it has been extensively studied.[8] There have been reports of its occurrence in a few other states and in Europe. No one knows of its possible occurrence in Pennsylvania since there have been no reported studies on the problem in this area. In the lake under question, fish have been found to contain larval forms, and birds, such as blue herons and kingfishers (common primary hosts of the adult fluke), are inhabitants of the lake area. To our knowledge a search has never been made for the appropriate intermediate snail host or the miricidial forms. Though the appearance of the skin lesions in our patient could possibly resemble that of schistosome dermatitis, it is not believed that this was the etiologic agent. Cercariae were not seen in microscopic examination of water samples or on the vegetation mentioned previously. However, even more significant, there have been no other instances of dermatitis reported in other bathers swimming in the same water at the same time. Schistosome cercariae when hatched in crops attack the skin of practically all individuals with whom they come in contact.

Dermatoses have been reported from contact with marine animals; hydroids,[9] coral,[10] and the tentacles of the Portuguese man-of-war.[11] Sams[12] has reported on the occurrence of an acute urticarial-papular eruption found among sea

bathers along the southern coast of Florida. The exact etiology has not been determined but it has been speculated that "Seabather's eruption" may be due to some living, microscopic organism, in the nature of nematocysts from some form of coelenterate which is free-floating, or perhaps from some unicellular organism equipped with similar stinging apparatus.[12] However, it is noteworthy that Ayres[13] has called attention to a patient with this dermatitis who reported that the initial onset of the pruritus occurred in some bathers on the Gulf Coast at the time of the "red tide." This is of particular interest in that salt water algae were found responsible for this discoloration of the sea water.[14] In further considering dermatitis found in swimmers it is noteworthy to point out the existence of another type of fresh and salt water algae, the diatoms. These are unicellular and produce shells of pure opalescent silica ($SiO_2 - nH_2O$).[15] Theoretically contact with sufficient numbers of these algae encased in siliceous shells might prove to be mechanically irritating to the skin. However, our plankton sample did not reveal any diatoms.

The question then arises as to the possibility of these blue-green algae getting into general water supplies and causing allergic dermatitis from contact with water in bath tubs, showers, wash basins, laundries, etc. The latter part of this question can be only a matter of speculation, but the entrance of such algae into the general water supply is a fact and depends largely upon the particular type of reservoir supply involved. Anabenae are inhabitants of lakes, ponds, and other types of lentic bodies of water and are absent from rivers, creeks, and fast-moving streams. Even when the initial water supply from a river is temporarily stored in an artificial reservoir, conditions are still not conducive to the growth of algae, but when the river water is diverted to a natural lake prior to purification processes then algae find more favorable environmental conditions for their growth. However, when the algae-containing water flows over the top of the reservoir wall and from there into a fast-moving stream, before the algae have traveled 100 yards downstream they disappear from the water content.[16] Within this short distance of transit they are removed by catching on and clinging to rocks, plants, and branches protruding within the stream bed and are subjected to traumatic fragmentation exerted by mechanical factors involved in swift moving currents. Blue-green algae are largely inhabitants of surface water so that if the water supply is obtained through pipes draining from the deeper portions of the reservoir, this water will generally be free of any growth of algae. The usual water purification procedures such as settling and chlorination do not affect the presence of algae and thus these unicellular plants have on occasion been found filtered from tap water. However, whether or not Anabena or other blue-green algae in question actually survive conditions of transport from the reservoir and whether they or their chemical products of disintegration are actually found in our own supply of tap water is a matter under current investigation.

## SUMMARY

1. A case history is presented in which a child developed an erythematous-papulo-vesicular dermatitis of the exposed portions of the body on swimming in a lake containing a "water bloom" of blue-green algae.

2. The patient was found to have an allergic contact type dermatitis due to cutaneous sensitivity to phycocyanin, the blue pigment found in Anabena.

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# THE SCHULTZ-DALE TECHNIQUE

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I AM glad for this opportunity to discuss the Schultz-Dale technique because I believe this method is capable of becoming a more generally useful tool than it has been in the past.

The Schultz-Dale technique dates from 1910 when Schultz[1] found that if an intestinal strip from a sensitized guinea pig was excised and mounted in a bath of oxygenated saline solution, addition of the specific antigen to the bath would induce a contraction of the tissue. Dale[2] later modified the method by substituting the uterine muscle of the virgin female guinea pig in place of the intestinal strip, and so the method came to be called the Schultz-Dale method.

Since the contraction elicited by the antigen in the sensitized muscle is initiated by reaction of antibody with antigen, the specificity of the Schultz-Dale reaction is the same as that of other serologic reactions. In many instances the Schultz-Dale method has the advantage of greater sensitivity than other immunologic techniques but lacks the advantage of the quantitative features of some of the other methods—the precipitin reaction, for example. However, as I shall show later, the Schultz-Dale reaction can be adapted to semiquantitative work and provides a rapid, simple, and highly specific method for determining antigenic properties.

In this discussion I shall describe our Schultz-Dale apparatus which contains several features I have never seen described in connection with the Schultz-Dale test. I shall also describe the saline solution we use, the methods of sensitizing guinea pigs for Dale tests, and our adaptation of the Schultz-Dale tests for quantitative work. Lastly, I shall show you some practical applications of the test for solving different problems of the allergist, immunologist, and protein chemist.

## SENSITIZATION

In preparing guinea pigs for Schultz-Dale tests the method of sensitization and the size of the sensitizing dose will depend upon the character of the antigen and upon the objective of the experiment. For an antigen such as egg albumin we have found that the sensitivity of the excised uterine muscle varies directly with the size of the sensitizing dose—the larger the dose the more sensitive the muscle will be. If you are interested in specific sensitization to a single antigen, however, there are advantages in using small sensitizing doses.

Those of you who have had experience in the preparation of protein antigens are aware of the difficulties of obtaining a serologically homogeneous prep-

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Presented at the Symposium on "Techniques of Experimental Immunology Applied to Allergy" in the Postgraduate Course of the American Academy of Allergy, Feb. 15, 1952.

Received for publication May 11, 1953.

aration. For example, an ovalbumin preparation that has been recrystallized 3 or 4 times will still contain serologically detectable traces of conalbumin. Injection of moderately large doses (1 mg.) of this preparation will sensitize guinea pigs both to ovalbumin and to conalbumin. Nevertheless, such a preparation can be used to induce specific sensitivity to ovalbumin in guinea pigs providing that the sensitizing dose is large enough to exceed the minimal sensitizing dose for ovalbumin, but too small to exceed the minimal sensitizing dose of the conalbumin contaminant. This feat can be accomplished quite readily in the guinea pig, in which a high degree of sensitivity can be induced by a single sensitizing injection.

On the other hand, if your desire is to sensitize to a complex of antigens such as, for example, an animal serum, and you want to develop sensitivity to all components of the complex, it is advisable to use a relatively large sensitizing dose or to enhance the sensitizing activity with an adjuvant. The sensitizing effect of a large dose of antigen will be greater if the dose is divided and injected in series, say, on the first, third, and fifth day. We find it more convenient, however, to use an adjuvant. Then a single injection will usually suffice to sensitize to individual components of a mixture. The alum precipitate, for example, enhances the sensitizing activity of ovalbumin more than 400-fold.[3] For weak antigens such as the bacterial proteins or bacterial protein-polysaccharide combinations, Freund and associate's[4] mineral oil emulsion is even more effective than the alum precipitate.

Sensitization may also be established by passive transfer, for which intravenous injection of the antiserum is the preferable method. Guinea pigs sensitized by intravenous injection may be used for Schultz-Dale tests within twenty-four hours. Guinea pigs sensitized by the intraperitoneal route should not be used until forty-eight hours after injection in order to permit development of maximal sensitivity. The chief advantage of active sensitization is that the uterine muscles of actively sensitized animals are manyfold more sensitive than those of passively sensitized animals. This may be an important factor when you are attempting to detect trace amounts of a specific antigen concealed in a complex mixture.

### INCUBATION PERIOD

Another important factor to be considered in the development of sensitivity is the length of the incubation period. Many people are inclined to cut this period too short. The peak of maximal sensitivity in smooth muscles does not coincide with the peak of maximal sensitivity as determined by gross anaphylaxis, but appears some time later. For example, guinea pigs sensitized with ovalbumin show their highest gross sensitivity at 2 weeks, but the smooth muscles from these animals show their highest sensitivity at 3 weeks. Once maximal smooth-muscle sensitivity has been attained it apparently remains fairly constant for a period of eight months or more.

The time required to develop maximal sensitivity in smooth muscles will vary also with different antigens. For example, serum albumin requires a longer incubation period than ovalbumin. The bacterial antigens also require a long incubation period, sometimes as long as eight weeks.

### SALINE SOLUTION

The selection and composition of a saline solution for bathing the muscle tissues in the Schultz-Dale test is important enough to deserve some consideration in this discussion. Even under optimum conditions a significant proportion of uterine muscles shows persisting spontaneous contractions and these muscles are not suitable for Dale tests. The proportion of muscles showing this hyperirritability can be reduced to some extent by selection of the saline solution. We prefer Tyrode solution, particularly for muscles from animals weighing more than 300 grams. The irritability can be further diminished by reducing the calcium chloride concentration of the Tyrode solution. Unfortunately, however, when the calcium concentration is reduced the sensitivity of the muscle to antigen and to histamine is also diminished. When calcium is completely removed the sensitized tissue will not respond to antigenic stimulation. We have found that Tyrode solution containing 0.05 gram calcium chloride per liter is most favorable for conditioning the tissue to a high degree of antigenic sensitivity and results in a low proportion of hyperirritable muscles. With this solution we have successfully tested uterine muscles from guinea pigs up to 1 year of age.

### SCHULTZ-DALE APPARATUS

Our Schultz-Dale apparatus duplicated one designed by L. C. Miller and G. Woodard and used by them in the Division of Pharmacology of the Food and Drug Administration for the quantitative evaluation of certain spasmolytic drugs. The apparatus was publicly demonstrated[5] but no detailed description has ever been published.

Fig. 1 shows details of the muscle bath which is a modification of one designed by Harne.[6] The uterine muscle is suspended in chamber *A*. This muscle chamber is connected by two side arms to an aeration column, *B*, the lower portion of which is constricted. Oxygen enters at the lower end of the aeration column through the capillary tubing on the right. In use, of course, the bath is filled to the 50 ml. mark with Tyrode solution, which is about at the level of the solid glass brace. Oxygen bubbling up through the solution in the constricted portion of the aeration column causes the solution to circulate in a counterclockwise direction and promotes gentle but thorough mixing of the solution in the muscle chamber. The test agent is added at the top of the aeration column and is rapidly mixed and carried into the muscle chamber. The tube on the left is connected to a two-way stopcock for emptying the bath and refilling with fresh Tyrode solution.

Fig. 2 is a schematic diagram of the complete apparatus. This consists essentially of two 50 ml. muscle baths mounted side-by-side in a water bath maintained at 38° C. The two horns of a uterus are tested simultaneously, with levers arranged so that their tracings appear one above the other. The uterine muscles are attached to the silk threads by means of hooks fashioned from platinium-iridium wire (B & S 30 gauge). The points of the hooks are sharpened and are quickly and easily passed through the ends of the muscle strip.

The large reservoir on the left contains Tyrode solution under gravity pressure for filling the baths with solution at bath temperature.

The levers—with the catenary chains detached—are counter balanced by lead weights. With chains attached and the levers in horizontal position a force of 1.4 Gm. is exerted on the muscle end of the lever. We do not vary this force from tissue to tissue.

The writing point is fashioned of light aluminum wire and is swiveled on a pin through the muscle lever so that the writing point can be turned back and away from the writing position when desired. This is illustrated by the top lever in Fig. 2.

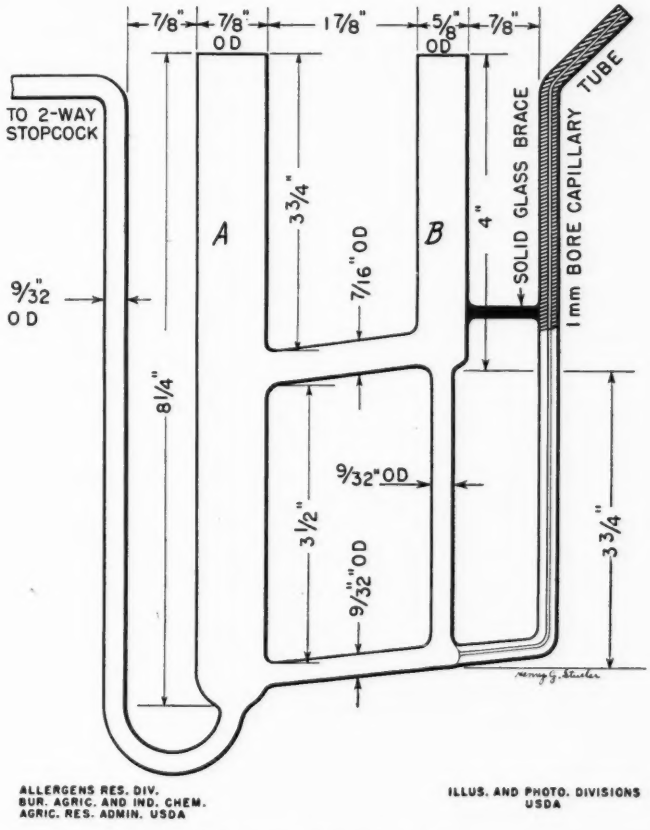


Fig. 1.—The muscle bath.

The kymograph paper travels 3 mm. per minute. This is a slower rate than most people use, but permits us to make many more tests on a given length of paper. We use a long-paper kymograph which provides 10 linear feet of smoked paper for tracings. When the tracings for one or more experiments are completed, a 35 mm. camera is positioned to photograph these data at 20:1 reduction on Microfile copy film. The kymograph paper is finally discarded since a permanent record of the tracings is preserved on negatives of convenient size for

filing. From these negatives enlarged prints may be made directly on lantern slides or on paper of desired size for notebooks or for publication, without loss of essential detail.

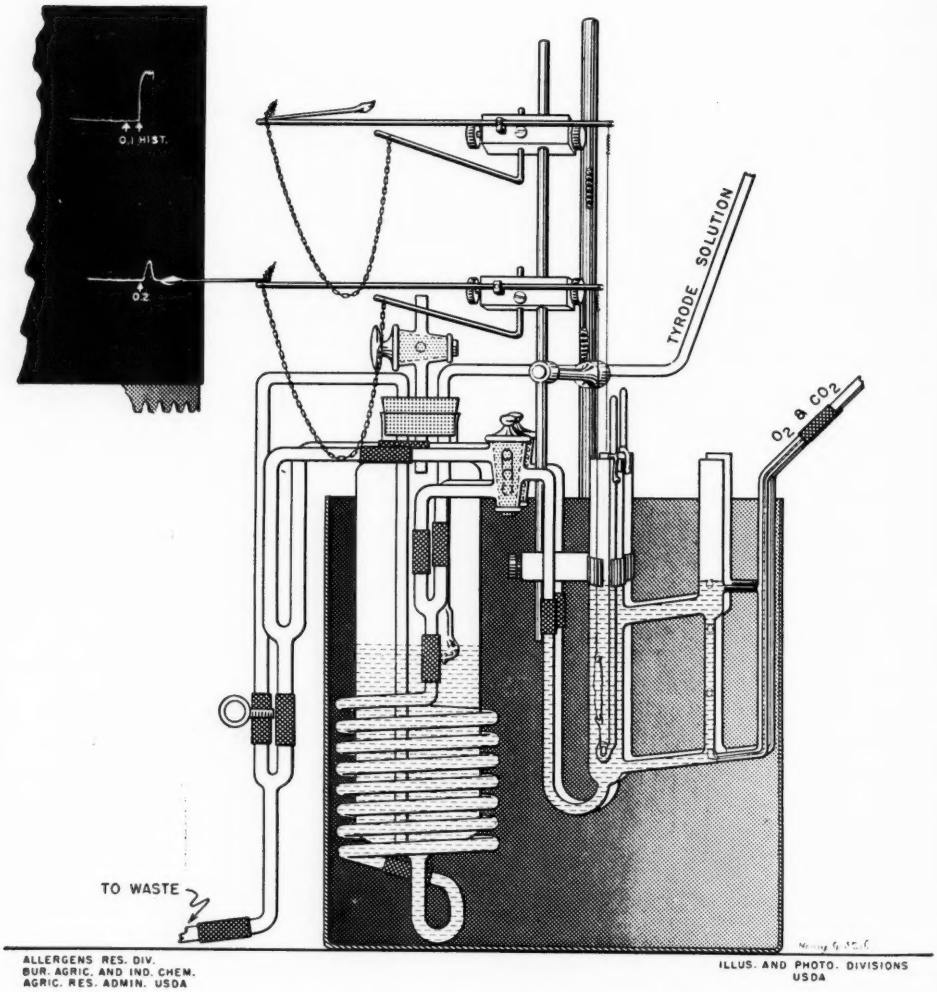


Fig. 2.—Schematic diagram of the Schultz-Dale apparatus.

## SENSITIVITY AND SPECIFICITY OF THE SCHULTZ-DALE REACTION

One of the important advantages of the Schultz-Dale technique is its extreme sensitivity. This property is of especial value in detecting and identifying very small amounts of contaminants. A highly sensitized uterine muscle will respond to a dose of 0.005γ of ovalbumin. A majority of sensitized tissues will respond to doses of 0.05γ or less. The limit of sensitivity of the precipitin test is about 1γ ovalbumin. Hence, the Schultz-Dale method is from 20 to 200 times more sensitive than the precipitin test for ovalbumin. For detecting the castor

bean allergen with specifically sensitized guinea pigs the Schultz-Dale method is at least 200 times more sensitive than detection by gross anaphylactic shock and 6,000 times more sensitive than detection by anaphylactic sensitization.[7]

The initial anaphylactic response in excised smooth muscle of the guinea pig shows the same degree of specificity as other antigen-antibody reactions. In general, desensitization of the isolated tissue is also specific. If an animal is sensitized to two antigens and the uterine muscle is desensitized to one of them, the tissue will still respond to test with the second antigen. Occasionally, however, desensitization of a multiple-sensitized muscle with a strong antigen will suppress subsequent response to a weaker one.

Relatively few purified proteins, including crystalline preparations, are serologically homogeneous. Accordingly, in the comparison of protein preparations from the same biologic source, one should be mindful of reactions caused by contaminants. Contaminants may be detected in several ways. One method involves sensitizing guinea pigs with a purified preparation of the suspected contaminant and using the uterine tissue to test for the contaminant. Likewise, uterine muscles from animals sensitized with the preparation may be tested directly with the suspected contaminant.

Evidences of contamination may also be revealed with the Schultz-Dale method by conducting comparative tests at different dosage levels. Thus, two homogeneous preparations of the same preparation will induce like responses at all dosage levels. If one preparation is contaminated, the two proteins may still appear to be identical when tested at low levels, but will appear to be dissimilar at high levels. Conversely, two different proteins containing a common trace contaminant may cross-react only at high dosage levels.

## QUALITATIVE TESTS

To carry out the Schultz-Dale test, a sensitized guinea pig is killed by a blow at the base of the skull; the two uterine horns are removed and dropped into a beaker of warm, oxygenated Tyrode solution. Each horn may then be cut transversely into 2 to 4 strips, depending upon the number of tests to be conducted. For qualitative tests we usually cut each horn into 2 strips. One strip from each horn is mounted in each of the 2 muscle baths and the 2 remaining strips are stored in oxygenated Tyrode solution. When first mounted in the bath and connected to the writing lever the muscle strip is in clonic contraction. Resting for one-half hour is usually sufficient to insure complete relaxation of the muscle, which is essential for uniform antigenic response. Relaxation can often be hastened by occasional flushing and renewal of the bath. This apparently removes some active constituents that have been released from the tissue cells during manipulation and sectioning of the muscles. When the muscles are completely relaxed and the writing point has been tracing a uniform base line for ten minutes, the antigen is introduced and the response is recorded.

Practical examples of the use of the Schultz-Dale method in qualitative tests will be given later.

QUANTITATIVE TESTS

I mentioned earlier that the Schultz-Dale method is adaptable to semi-quantitative work. In the development of any quantitative biologic method you must have some means of measuring response and be able to show some definite correlation between the magnitude of the response and the size of the test dose.

Fig. 3 illustrates the relationship between the test dose and the response. The tracings in Fig. 3 record the responses to different doses of ovalbumin of 6 uterine strips from a guinea pig that had been sensitized to ovalbumin. The response for a single test dose is calculated by determining the numerical ratio of the height of the contraction caused by the test dose of antigen to the height of the contraction caused by histamine (100γ). The dosages used here covered the range from zero response to a response of 0.76. Naturally, only one test can be made on each strip because the first dose of antigen would partially desensitize to subsequent doses.

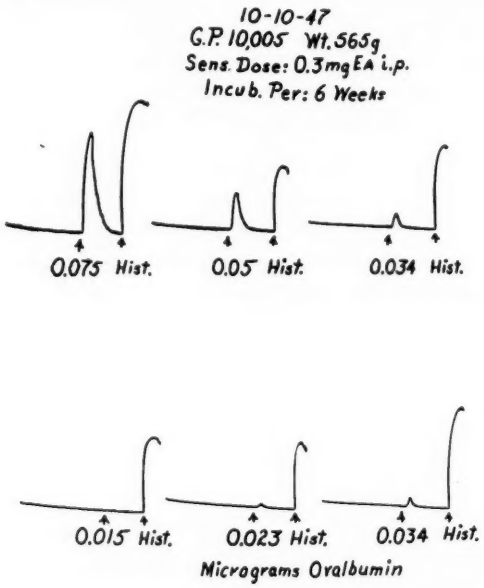


Fig. 3.—Relationship of dose to response in sensitized uterine muscle.

Now, if you plot the dose, or some function of the dose, against the response, you get what is called the dosage-response curve. Fig. 4 shows the dosage-response curves for ovalbumin with uteri from different groups of animals sensitized with ovalbumin. We observed that when the logarithm of the dose was plotted against the contraction-response we got the sigmoid-shaped curve typical of so many biologic reactions. The results in Fig. 4 show the effect of the sensitivity of the uterine muscle on the slope of the dosage-response curve. There was a 70-fold difference in the sensitivity of uterine tissue from animals in Group A as compared with those in Group F, and excepting Group E, the slopes were essentially parallel. We concluded, therefore, that the slope of the dosage-response curve was independent of the sensitivity of the tissues.

We also found that the mean slope of dosage-response curves for ovalbumin was essentially the same as those of such diverse antigens as Flaxseed-1A (allergenic protein from flaxseed) and tuberculoprotein from the tubercle bacillus. Hence, the slope is independent of the type of antigen.

From the data recorded in Fig. 4 it may be observed that the portion of the curves lying between contraction-responses of about 0.2 and 0.7 is essentially a straight line and can be represented by the linear equation

$$Y = a + bx$$

Where Y is the observed response caused by a test dose whose logarithm is x; a is a constant, and b equals the slope of the line. Under the conditions of our experiments the mean-slope of the dosage response curves was 2.0.

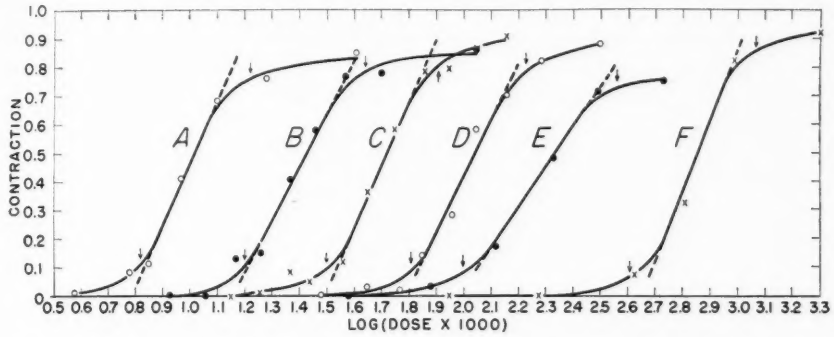


Fig. 4.—Effect of sensitivity of uterine muscle on the slope of the dosage-response curve.

With the above relationship established it is apparent that we may now calculate the dose that will produce some standard response—say a contraction-response of 0.40 or 40 per cent. We call this standard, 40-per cent contractive dose the $CD_{40}$. To calculate the $CD_{40}$ we select from our experimental results a test dose that produced a response somewhere between 0.2 and 0.7. The $CD_{40}$ is then calculated from the simultaneous equations

$$Y = a + bx_1$$
$$0.40 = a + bx_2$$

where $x_1$ is the logarithm of the dose that caused Y response and we wish to solve for $x_2$, the logarithm of the dose that would produce a response of 0.40. By subtracting the equations, we get

$$Y - 0.40 = bx_1 - bx_2$$

which reduces to

$$x_2 = \frac{bx_1 + 0.40 - Y}{b}$$

where b equals 2.0 under our experimental conditions. Since the value we obtain for $x_2$ is the logarithm of the dose that would produce a contraction-response of 0.40, the $CD_{40}$ is the antilog of $x_2$.*

In applying these principles to quantitative tests, we sensitize a guinea pig with the specific antigen we wish to test for. After the prescribed incubational

*We are indebted to F. M. Wadley for suggesting this method of calculation.

period the uterus is removed and each horn is cut into 3 or 4 strips depending upon the length of the uterine horns. Half of the strips are used to determine a submaximal reactive dose of the purified antigen to which the animal was sensitized and the remaining strips are used to determine a submaximal reactive dose of the unknown mixture. We then select an observed response for both the reference antigen and the unknown mixture that lies between 0.2 and 0.7 and calculate the $CD_{40}$ for each preparation. The quantitative relationships of the activity of the two preparations may be readily determined from these two results.

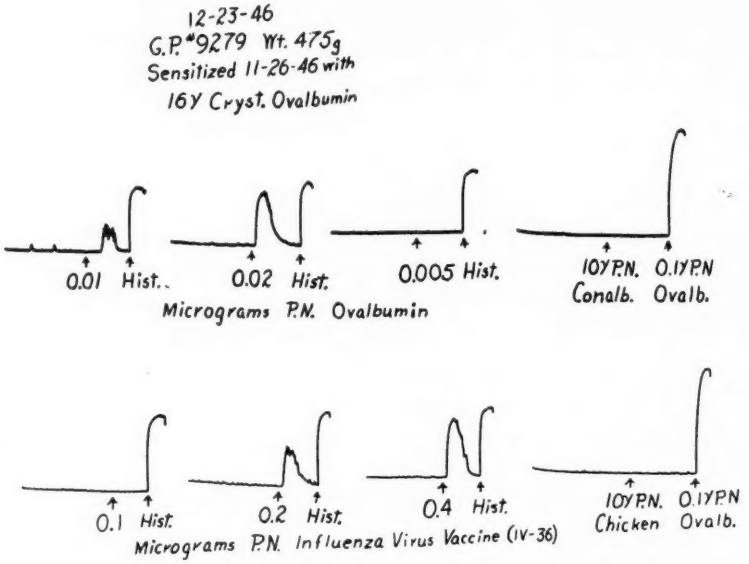


**Fig. 5.**—Determination of ovalbumin in influenza vaccine.

We have observed that the sensitivity of the uterine strips is not entirely uniform throughout the length of the horn. The end sections are usually somewhat more sensitive than the intermediate sections. Uniformity of sensitivity was not improved by storage of the tissues for one hour or more before use as suggested by Moody.[8] However, corresponding sections of the two horns exhibit about equal sensitivities. In performing the assay, therefore, it is advisable to match corresponding sections from the two horns, using one for a test of the reference antigen and the other for the unknown. The accuracy of the calculated $CD_{40}$ will be improved, also, if it represents the average computed from two or more observations. However, with only 3 or 4 strips available for each test it is not always possible to obtain more than one response within the limits of 0.2 and 0.7.

Fig. 5 shows a practical application of our quantitative technique. The problem here was to determine the presence, or prove the absence, of egg white protein in influenza vaccine. The vaccine was prepared from virus that had been propagated in incubating eggs and supposedly separated from egg proteins

by super-centrifugation. This guinea pig was sensitized with 16γ of crystalline ovalbumin—a small dose to insure specific sensitivity to ovalbumin. The first 3 strips on the top row were titrated with different doses of ovalbumin and the first 3 strips on the bottom row were titrated with the vaccine. The vaccine contained 30γ/ml. of protein nitrogen. This experiment showed that about 5 per cent of this protein nitrogen was identifiable as ovalbumin. The last 2 strips in this experiment were used to demonstrate the specific sensitivity of the tissue to ovalbumin. Neither chicken-muscle extract nor conalbumin induced a reaction in the sensitized tissue.

## PRINCIPLES ILLUSTRATED

Perhaps the best method of illustrating the various principles of the Schultz-Dale technique is to demonstrate its use in the solution of a specific problem. For this purpose I have selected an experiment conducted recently in collaboration with our Eastern Regional Research Laboratory.[9]

Polis, Shmukler, and Custer[10] recently isolated a crystalline albumin from milk that exhibited physical and chemical properties identical with blood serum albumin. Our problem was to determine whether the two proteins were serologically identical. At first glance this would seem to be a very simple problem and would require only a couple of cross-reaction tests by the Schultz-Dale method. However, a slight contamination in the milk albumin preparation caused the problem to be more difficult than we anticipated.

The first thing to do in a problem like this is to find out whether each of the proteins will react with the antibodies produced by the other protein and, secondly, to determine whether the antibodies are neutralized in the process. Fig. 6 shows such a test. In this experiment the animal was sensitized with serum albumin. Serum albumin induced a contraction in the first horn of the uterus and neutralized the antibodies to itself and to milk albumin. In the corresponding segment of the second horn of the same uterus, milk albumin induced a reaction and desensitized the tissue to serum albumin. This experiment was repeated at dosage levels of 1, 10, and 100γ of protein nitrogen of the two preparations. All gave the same results. These results showed that the preparations of milk albumin and serum albumin used contained a protein in common.

Similar experiments were conducted with uterine muscle from guinea pigs sensitized with crystalline milk albumin. Reaction to milk albumin desensitized the muscle to serum albumin at all dosage levels. Likewise, when the tests were conducted at doses of 1 or 10γ of protein nitrogen, serum albumin reacted and desensitized the tissue to milk albumin. However, when tests were made with doses of 100γ of protein nitrogen, the serum albumin did not completely desensitize to milk albumin. These results implied that the two preparations contained a common protein and that the milk albumin contained, in addition, a small amount of a protein not found in the serum albumin.

The foregoing experiments indicated that the major components of the two protein preparations were probably identical. We then conducted some

quantitative comparisons of the two proteins to make sure that the cross-reactions were not caused by some minor component of one of the preparations. Fig. 7 shows a quantitative comparison of the activity of serum albumin and milk albumin in the uterus of an animal that had been passively sensitized to serum albumin. In this test the milk albumin preparation was about 80 per cent as potent as the serum albumin.

TABLE I. ANTIGENIC POTENCY OF MILK ALBUMIN AS COMPARED WITH SERUM ALBUMIN

| METHOD OF SENSITIZATION | RATIO OF POTENCY MILK ALB./SERUM ALB. |
|---|---|
| Active | 0.80 |
| Passive | 0.80 |
| Passive | 0.82 |
| Passive | 0.82 |
| Passive | 0.89 |
| Active | 1.0 |
| Passive | 1.0 |
| Average | 0.87 |

Table I summarizes the results of seven quantitative determinations of the activity of milk albumin compared with serum albumin. All 7 animals were sensitized to serum albumin, 2 by active sensitization and 5 by passive sensitization. The results ranged from 80 to 100 per cent of equivalency and averaged about 90 per cent. This result is well within the range of error of the method.

This might well have completed our investigations of the serologic relationship of milk albumin and serum albumin. However, we were curious to see if we could confirm the presence of a contaminant in the milk albumin preparation and perhaps identify it. Fig. 8 shows the effect of large test doses on the reactions between milk albumin and serum albumin. This animal was sensitized with milk albumin. In the first horn of the uterus, milk albumin reacted and desensitized to serum albumin. In the second horn, serum albumin reacted but did not desensitize to milk albumin. This test showed that milk albumin contained an antigen not contained in serum albumin. Since this response (reaction with milk albumin subsequent to desensitization with serum albumin) showed up only on tests with large doses, and only in guinea pigs sensitized with the milk albumin preparation, we reasoned that it was caused by a contaminant in the milk albumin preparation.

$\beta$-lactoglobulin, another protein of milk whey, seemed to be a likely suspect so we tested the strips with $\beta$-lactoglobulin and both strips responded. We tentatively concluded, therefore, that the milk albumin contained an amount of $\beta$-lactoglobulin sufficient to sensitize animals to $\beta$-lactoglobulin as well as to the albumin.

The presence of $\beta$-lactoglobulin in the milk albumin was corroborated by tests on uterine muscles from animals sensitized with $\beta$-lactoglobulin. Positive results were always obtained when these tissues were tested with large doses of milk albumin. Similar tests with equal doses of serum albumin were always negative.

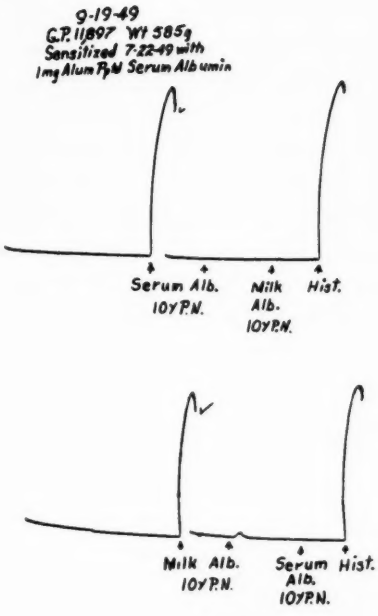


Fig. 6.

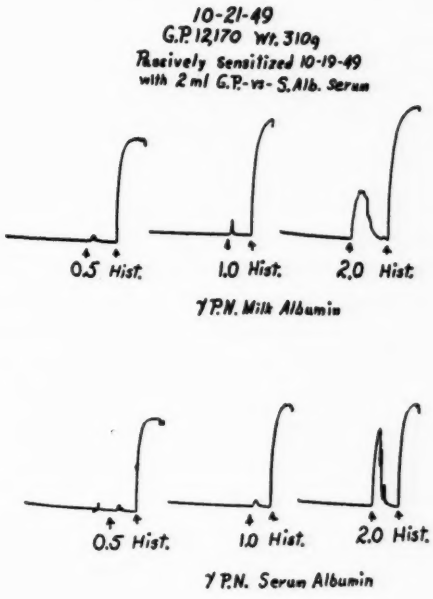


Fig. 7.

Fig. 6.—Antigenic relationship of serum albumin and milk albumin.
Fig. 7.—Comparison of antigenic activity of serum albumin and milk albumin.

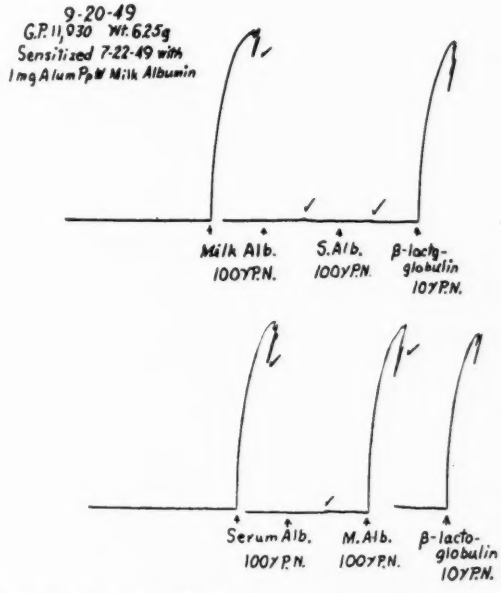


Fig. 8.—Detection and identification of a contaminant in milk albumin.

The concentration of β-lactoglobulin in the milk albumin was determined in 3 experiments by our quantitative method. In one experiment, the response induced by the β-lactoglobulin in a dose of the milk albumin containing 60γ of protein nitrogen was about equivalent to that induced by 0.011γ of protein nitrogen from the pure protein. This test indicated that the milk albumin contained about 0.018 per cent β-lactoglobulin. The other two tests showed 0.12 and 0.015 per cent.

We then looked for albumin in the β-lactoglobulin. To do this we used uterine muscle from an animal sensitized with serum albumin, the serological equivalent of milk albumin. The results in Fig. 9 show that 0.06γ of protein nitrogen of serum albumin gave a definite but submaximal contraction in the sensitized tissue. On the other hand, doses of β-lactoglobulin up to 1,600γ of protein nitrogen did not induce a contraction and these doses did not desensitize the uterus to subsequent reaction with 0.06γ of protein nitrogen of either serum albumin or milk albumin. The results of this experiment showed that the β-lactoglobulin contained less than 0.004 per cent of albumin, a really remarkable separation.

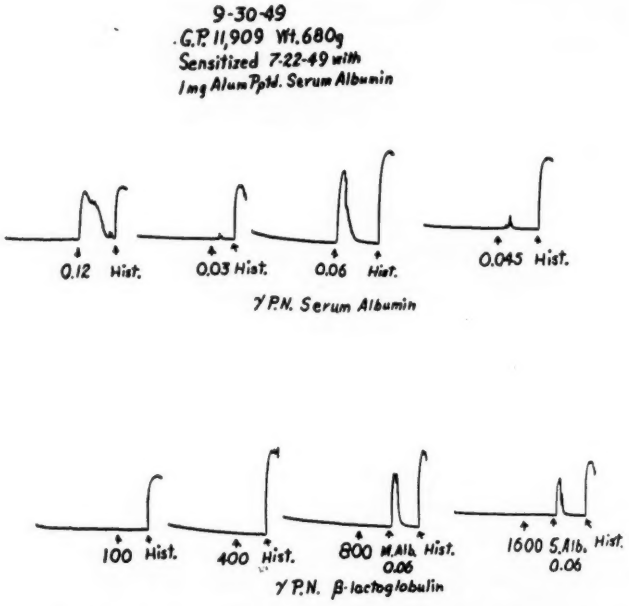


Fig. 9.—Determination of albumin in β-lactoglobulin.

The experiment recorded in Fig. 10 was designed to determine whether the milk albumin preparation contained contaminating proteins in addition to β-lactoglobulin. This animal was sensitized with milk albumin. The 2 uterine strips were first desensitized with β-lactoglobulin. Milk albumin induced a response in strip 1, and this reaction desensitized the tissue to serum albumin. In strip 2, serum albumin induced a reaction subsequent to desensitization with β-lactoglobulin, but reaction with the two preceding preparations did not

desensitize uterine tissue to the final test with milk albumin. These results showed that the milk albumin contained at least one contaminant in addition to β-lactoglobulin.

It is always advisable to compare your isolated protein with the original source whenever possible because of the possibility that the serologic properties may have been altered during the process of fractionation. For this reason we performed a few experiments with bovine serum and raw skim milk.

The experiments recorded in Fig. 11 reveal much useful information. This animal was sensitized with bovine blood serum. Accordingly, the uterine muscle from this animal should contain specific antibodies for each of the proteins in bovine serum. In this experiment we used 4 uterine strips. The pair at your left were tested at a dosage level of 10γ protein nitrogen and the pair on the right were tested at a dosage level of 100γ protein nitrogen. β-lactoglobulin failed to react at either dosage level. As a matter of fact, we have never obtained any evidence that protein identifiable with β-lactoglobulin appears in bovine serum. There have been a few reports in the literature indicating that it does.

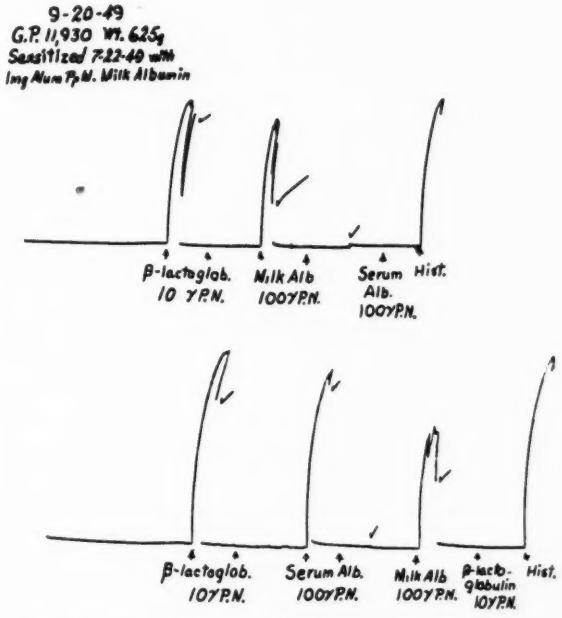


Fig. 10.—Detection of a second contaminant in milk albumin.

At the 10γ protein nitrogen dosage level, serum albumin (lower tracing) reacted and desensitized to milk albumin. Residual antigens of the bovine serum then induced a final, maximal response. In the top tracing, milk albumin induced a maximal response, subsequent to the negative test with β-lactoglobulin, and desensitized the tissue to serum albumin. However, the preceding tests did not desensitize the tissue to a final test with milk. The final response to

milk showed that milk contained another protein, common also to bovine serum, in addition to and distinct from crystalline milk or serum albumin.

When the same sequence of tests were repeated at 100γ protein nitrogen dosage level on the second pair of tissues a slightly different picture resulted. Thus, reaction with, and desensitization to, serum albumin did not desensitize to milk albumin. Now this reaction with milk albumin could not have been caused by the β-lactoglobulin contaminant because this tissue was not sensitive to β-lactoglobulin. Hence, the second contaminant in the milk albumin preparation is a protein common to both milk and blood serum. Other workers have shown that milk and blood serum contain serologically identical immune globulins. It is probable that the second contaminant in the milk albumin preparation is an immune globulin.

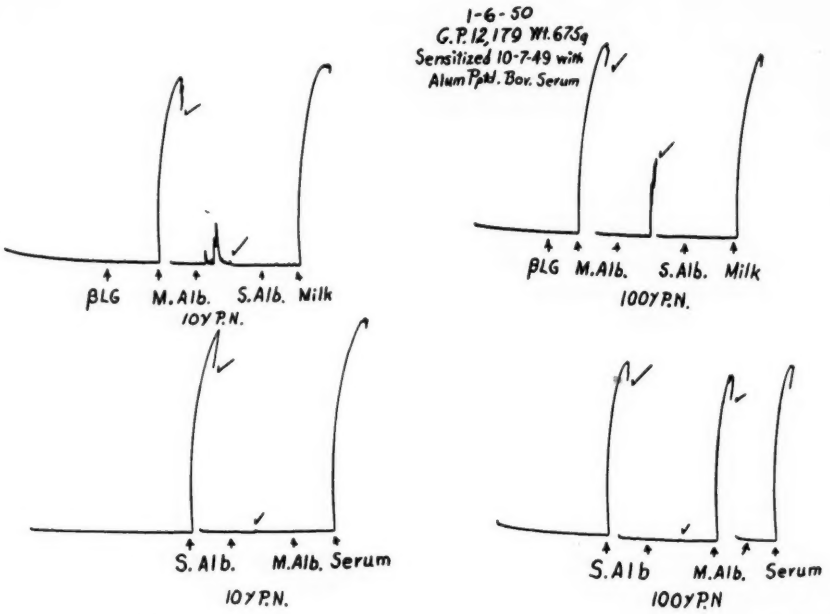


Fig. 11.—Demonstrating two proteins common to milk and blood serum and providing tentative identification of second contaminant in milk albumin.

The succession of related experiments I have just described utilized in a practical way the several essential principles of the Schultz-Dale technique. Additionally, I believe this series of experiments demonstrates the utility and potentialities of the Schultz-Dale technique for solving many of the problems encountered by the allergist, immunologist, and protein chemist.

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# Editorial

## SOYBEAN AS A MILK SUBSTITUTE FOR POTENTIALLY ALLERGIC INFANTS

IT is a manifestation of common sense not to expose a child who has a close family history of allergy continually or without necessity to well-known sensitizing agents. On the other hand, most of these children are exposed to many such allergens to which they do not become sensitized. There is evidently more to it than the mere fact of exposure.

The article by Johnston and Glaser in this issue is therefore in part founded upon logical premises and goes farther than any previous attempts at the prophylaxis of allergic disease in that the authors have actually followed their patients over a considerable period of time. They claim that withholding milk from the potentially allergic infant during the first few months not only prevents sensitization to milk, which is to be expected, but also prevents sensitization to other allergens, such as pollens, animal danders, and dust in later childhood. This is indeed an extraordinary finding and it is doubtful if it will find ready acceptance by most allergists. Furthermore, the feeding of a newly born infant on a soybean food can be a somewhat hazardous procedure and has the possibility of doing more harm than good.

The two soybean foods which are most commonly used (Mull Soy and Sobee) will nourish an infant as well as milk provided they are well tolerated. The trouble with these foods, however, has always been that there is something in soybean flour, at present unknown, which often causes loose bowel movements and sore buttocks, sometimes to the point of a severe diarrhea, and the younger the infant the more likely is this to take place. Diarrhea in a newborn infant is no light matter and most pediatricians would not be willing to risk it unless the feeding of a soybean food were proved a necessity by existing evidence of milk allergy. It therefore seems unlikely that the procedure advocated by Johnston and Glaser will be widely used.

Allergy to milk is certainly not uncommon in infants and can be responsible for a variety of symptoms. At present it is being overemphasized, and while the milk-free infant foods have a definite and a valuable place in the treatment of some allergic infants, they are being used altogether too much in many situations which have nothing whatever to do with milk allergy.

L. W. H.

# American Academy of Allergy

## Announcement

The Local Committee on Arrangements for the Houston meeting of the Academy in February, 1954, has arranged with the National Railways of Mexico for a post-convention tour over their lines to Mexico. Preliminary plans indicate that this tour, which will be sponsored entirely by the railway for members of the Academy and their families, will leave Houston at 8:20 P.M., February 3, arriving in San Antonio at 2:00 A.M., February 4. On February 6, the tour will arrive in Mexico City. A short morning tour in Mexico City is scheduled for Saturday morning, February 6. Side trips are scheduled on February 7, 8, 9, and 10, covering Pueblo, Taxco, Cuernavaca, and other points of interest. A bull fight, of course, is worked in on the side. The tour will leave Mexico City Thursday, February 11 and will arrive in San Antonio, February 12, Friday, where it will disband.

# Announcement

## Canadian Academy of Allergy Meeting

The Canadian Academy of Allergy meeting was held June 16, 1953, at the Royal Alexandra Hotel, Winnipeg, Manitoba. The program follows:

9:30 A.M. Studies on Urinary Histamine. Drs. J. Mitchell, Charles F. Code, and George P. Logan, Mayo Clinic, Rochester, Minn.

10:00 A.M. to 12 noon Histamine Symposium:

1. Roles of Histamine in the Anaphylactic Reaction in Guinea Pigs and Rabbits, Dr. C. F. Code, Mayo Clinic Foundation, Rochester, Minn.
2. Liberation of Histamine and Other Substances During Anaphylactic Reactions in Dogs and Monkeys. Dr. L. B. Jacques, Professor of Physiology, University of Saskatchewan, Saskatoon.
3. Role of Histamine in Allergic and Anaphylactic Reactions in Man. Dr. Bram Rose, McGill University, Montreal.

2:00 P.M. Recent Advances in ACTH and Cortisone in Allergy. Dr. Bram Rose, Montreal.

2:30 P.M. Concepts on Mode of Action of Salicylates. Dr. L. B. Jacques and Dr. J. Lowenthal, University of Saskatchewan, Saskatoon.

3:00 P.M. Experience With Pollen Surveys in Manitoba From 1939 to 1952. Dr. C. H. A. Walton and Dr. M. G. Dudley, Winnipeg.

3:30 P.M. Recent Advances in Bacterial Allergy. Dr. K. A. Baird, St. John, N. B.

4:00 P.M. Clinical and Experimental Allergic Cephalalgia. Dr. Jacques Leger, Montreal.

4:30 P.M. Effect of Antihistamines on Smooth Muscle. Dr. T. H. Aaron and Dr. W. Stewart, Edmonton.

## Course in Allergic Diseases

A course in allergic diseases will be given Oct. 19 to 29, 1953, at the Hôpital Broussais, 96 rue Didot, Paris XIV, France.

It will consist of lectures and practical exercises. Experimental anaphylaxis, experimental asthma, food allergy, allergic dermatitis, and the protective action of antihistamines will be covered. There will also be a theoretical and practical demonstration on the chemistry of antigens and antibodies and their dosage. Lecturers will also discuss migraine, allergies of the nervous system, serum and drug hypersensitivity. Practical demonstrations of the Schultz-Dale technique and aerosols in the treatment of asthma will be given, as well as a discussion of the treatment of allergic states with specific desensitization and antihistaminics.

Prof. Pasteur Vallery-Radot will conduct the course assisted by Dr. Halpern and others. The number of students is strictly limited. Applications should be addressed to Clinique Médicale, Hôpital Broussais, 96 rue Didot, Paris XIV, and accompanied by a check or money order, 5,000 francs for the theoretical course and 8,000 francs for both the theoretical and practical.

# Book Reviews

**A Manual of Clinical Allergy.** By John M. Sheldon, M.D., Robert G. Lowell, M.D., and Kenneth P. Matthews, M.D., Philadelphia and London, W. B. Saunders Company, 1953. 413 pages.

Most of the available text and reference books on allergy attempt to compress into a single volume the entire theoretical background of the subject in immunology, pathology, physiology and heredity, the technical details of diagnostic and therapeutic procedures, and a smattering of practical botany and mycology. With the rapid addition of both theoretical and practical material in the fields of histamine and the antihistamines, endocrinology and the steroid hormones, this feat of condensation into a book of convenient size is increasingly formidable. The authors of the present volume have recognized that the allergist now needs more than one book, and have confined their efforts to the practical and technical aspects of allergy, leaving to others the involved discussions of antibodies, histamine, animal anaphylaxis, and hereditary factors. The result is handy compilation of material useful to both the beginner in the field and the experienced allergist.

For the novice, the book starts appropriately with a detailed account of taking the allergic history and evaluating the physical status of the patient, progressing through skin testing to treatment, both specific and symptomatic. An appendix deals with other important problems of the embryo allergist: the choice of a location (preferably in an area serving a population of at least 25,000), planning the layout of an office suite, and the minimum equipment necessary, even to the furnishings of the waiting room. Advice on these points is specific and practical.

The experienced allergist will also find much material for which he has occasional need but does not use often enough to keep it constantly in mind. Much of this material is collected in tabulations or lists for ready reference. For example, the antihistamine drugs with their many trade names are listed in essentially complete detail, and the compositions of many other proprietary preparations used in allergic diseases are tabulated. Other useful compilations are the lists of possible etiologic agents of contact dermatitis in various portions of the body, the recommended concentrations of various contactants for patch testing, and prescriptions useful in the topical therapy of allergic dermatoses. Elimination diets and recipes for use in conjunction with them are covered in detail. Data on pollens are well supplied by O. C. Durham and those on molds by C. M. Christensen and M. A. Swaebly.

While the authors intentionally avoid the more controversial subjects, there are, as in all books on allergy, points with which others will disagree. Many would feel that the scale for reading scratch tests, in which an erythema 21 mm. in diameter without a wheal is classed as two plus and an erythema with wheal as three plus, is too liberal and would question the statement that intracutaneous tests are contraindicated when reactions of this intensity are obtained by scratch tests. Even more open to question is the statement that in passive transfer, the same site may be used over and over again at intervals of a day or two, testing with different antigens. The dosage schedules for hyposensitization follow the common but illogical practice of increasing the dose of each dilution arithmetically so that a 10 per cent increase from 0.45 c.c. to 0.5 c.c. of one dilution is followed by a 100 per cent increase from 0.05 c.c. to 0.1 c.c. of the next stronger. If expressing dosage in units has no other advantage, it should at least make it more obvious that either the 10 per cent increase is needlessly conservative or the subsequent 100 per cent increase unjustifiably dangerous.

In other cases, emphasis rather than specific statements might be criticized. For example, in the discussion of treatment of status asthmaticus, Isurprel and aminophyline are considered "specific medication," while cortisone and corticotropin are relegated to the category of "other adjuncts."

Despite these minor points, the book as a whole is a well written and handy collection of useful material for the physician treating allergic diseases. While it lacks the background material for an initial training in the subject, it will remain useful for convenient reference even after long experience in the field and it is highly recommended on this basis.

**Contact Dermatitis.** By George Waldbott, M.D., Springfield, Ill., Charles C Thomas, 1953. 281 pages. Price, $8.75.

In the lay mind, the allergist is often glorified as a medical Sherlock Holmes who combines careful deduction with scientific methods to track down the offending agents in a multitude of human ills. Too often this illusion is shattered by the practitioner who relies on a large number of routine skin tests and various nonspecific medications as an alternative to deep thought. This book should help the physician to maintain and justify the patient's illusion, at least in relation to the field it covers, which is indeed a form of allergy in which logical analysis is most important.

The main theme of the book, the correlation of the patterns of distribution of dermatitis with the daily contacts of the patient, cannot fail to arouse the reader to examine his patients with greater care and to consider their environments with renewed interest, thereby practicing a better grade of medicine. This type of analysis has its most obvious possibilities in relation to dermatitis of the hands with their innumerable contacts with potential sensitizing agents. In those cases in which the dermatitis remains localized to a relatively small portion of the skin of the hands, the pattern of contact may lead directly to a logical suspect among a great variety of possibilities. If the book only stimulates new interest in contact dermatitis of the hands it will have served a useful purpose.

Dermatitis of other portions of the body surface and occupational dermatitis are also covered with lists of possible causative agents, but unfortunately without adequate indication of which may act as primary irritants or specification of proper concentrations for patch testing. Discussions of differential diagnosis are reasonably complete and the analyses of the causative factors in pruritus ani and vulvi should prove useful. The suggestions for therapy cover both symptomatic relief and prophylactic treatment of plant dermatitis.

On the whole it is a stimulating and practical little book.